VOLUME 10 NOVEMBER 1961 NUMBER 4

AUSTRALASIAN ANNALS OF MEDICINE

Journal of Internal Medicine of Australia and New Zealand

published by

THE ROYAL AUSTRALASIAN COLLEGE OF PHYSICIANS

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The journal is published quarterly in February, May, August, and November. The subscription rate is four pounds per annum (one pound per issue), payable in advance to The Royal Australasian College of Physicians, 145 Macquarie Street, Sydney. The price of a single back number is twenty-five shillings. Advertising rates may be obtained on application to Australasian Medical Publishing Company Limited, Seamer and Arundel Streets, Glebe, Sydney.

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A COUNTRY GROWING UP[1]

An English periodical, *Encounter*, has been publishing a series of articles on great cities of the world. The first, naturally, was on London, that world in itself. Paris, New York and Manchester followed straight after, and then there was an article on Sydney. Sydney won this place not merely as being big (and it was its sprawling, unrelieved bigness that oppressed D. H. Lawrence during his visit in the twenties), but as having a human and man-made as well as a natural physical character of its own, with some tradition and signs still readable of the struggles and aspirations of its history.

> It (Sydney) can never quite decide whether to be a thriving, bustling, Anglo-Saxon boom-town, a new Birmingham or Chicago, or a happy-go-lucky South Pacific city, an Australian Rio de Janeiro or a Buenos Aires. And always the Australian continent is warring against these alien conceptions, insisting on its rights and making the city hard and bare and violent like its own nature. It is an inconsistent city, where men in blue serge suits jostle against other men wearing nothing but a pair of shorts or a linen suit and a tropical shirt. It is a gay, pagan, boisterous, raffish city, full of oysters and beer and pretty girls in summer frocks and white sails on the harbour.

The fact that Australia in 173 years has developed a great city with a character of its own, distinctive enough to earn a place in a series on great cities of the world, prompts the thought that Australia is growing up, developing its own variant of the western European civilization of which it is a trustee. The countryside has its lore and its ways, but there can hardly be an active civilization, as we understand civilization, without a city large enough to support learning, art, science, music and drama, and without a people ready and able to spare time from the daily concerns of practical life and to spare resources for the creative and enduring things of the mind. Australia has reached a stage in her history where she has both, and in such measure as to encourage us to feel some stirrings of a truly civilized way of living.

What are other signs of a country growing up? One is the attitude of people to the natural setting in which their lives are passed and to the earth from which their sustenance is drawn. To the early settlers Australia seemed a harsh and unfriendly land, hard and dry, seeming to resent the efforts of European man to establish himself. It was only slowly that the land and the intruder became reconciled, and indeed it is only comparatively recently that Australians generally have shown a true responsibility towards the soil and the landscape of their country.

This slowly-waking attachment to Australia was a favourite theme of Australian historical novels in the 1930's and 1940's. The authors follow the changing attitude of a settler who comes to Australia with the single intention of acquiring wealth and, before it is too late, enjoying some years of civilized living back in England. He fights and subdues the unfriendly tract of the country he has acquired, strengthened by the hope that his

[1] By the Professor of Early Literature and Language in the University of Sydney.

dream may be realized. But in time he begins to feel the country claiming him. He has a wife and family, sees his work continuing in a tradition he has founded, finds a deep satisfaction in what has come of the work of his own hands. An aggressive selfishness turns to a grateful attachment and willingness to make acknowledgement. The ambition fades, and, when he could gratify it, he makes no move.

The story in its numerous variations wore a little threadbare, but it reflects a theme that runs through Australian history. It is no exaggeration to say that the soil and man's use of the soil are the bases of settled civilized life. In older civilizations the observer is struck by the respectful tending of the soil, the source of all sustenance. The land is rhythmically rested, its fertility is yearly renewed by the spreading of manure accumulated on the straw-covered floors of stables during the winter. Fear of what might follow if the fertility of the soil should fail is transmuted into a respectful and grateful husbandry.

We still have with us the reminders of indifference and selfishness in the treatment of the soil in eroded slopes and gravelly expanses where the topsoil has been lost ; and these are equally reminders of the time that had to pass before a change of attitude took place. This change in the general attitude towards the soil of the countryside went along with a deepening imaginative acceptance and awareness of its beauty. By comparison with the brighter and more varied colours of the European countryside, the Australian is mostly subdued and monotonous. The great expanses of the plains at first seemed lonely and unfriendly to people accustomed to the valleys which, in England, seem to enfold their people protectively and affectionately. The early poets looked for the beauty their eyes had been accustomed to enjoy and beauty that suited the romantic mood, and found little of it. It was some time before the dignity and beauty of the more monotonous Australian landscape was accepted, was directly observed and was made the theme of poetry and art. It was only with effort and time that Australians became imaginatively and artistically at home in the new environment.

One of the most reassuring signs of responsibility towards the land appears when man, in his massive engineering works, takes serious thought for their effect upon the landscape. The Snowy Mountains Scheme brings great forces of nature under control, diverting rivers in their courses, drowning whole valleys, checking and releasing the gigantic energy of falling water. But care is taken that the contractors leave no litter behind. The construction camps and depots are removed, and if it is necessary to leave behind any sign (a massive concrete bed for machinery, for example) that man has intruded with temporary and alien structures, grasses and creepers are set growing over it. Access roads to new work sites are first scraped round the mountain flanks by bulldozer blades, in the beginning an unsightly scar in solitudes till then undisturbed. But vegetation is set growing to cover the scar, and the brown gravel road is replaced by the less conspicuous black ribbon of asphalt. Man has intruded roughly into the grandeur of these ancient solitudes and dealt violently with the courses followed for ages by great volumes of water. But when he finishes his work and leaves, the only signs of his intrusion will be the dams, the elegant concrete arch, the massive concrete or earth and rock filled dam, the roads inviting visitors to enjoy the grandeur of the valleys, and the pylons, like stylized human figures with upraised arms, passing the power lines from one to another across the valleys and over the crests of the hills.

The profession of medicine has contributed significantly to the reconciliation of man with the Australian environment that imposes isolation and loneliness on a dispersed population. The annals of medicine include the careers of doctors who have practised their profession single-handed in remote and scattered country areas, feeling more heavily than their fellows in more populous centres, with colleagues and hospital services at hand,

the lonely responsibility for decision involving life and death. The Royal Flying Doctor Service is unique in Australia. It ensures that in the sparsely-settled outback, people are never more than three hours away from a doctor, and that in emergency an interim diagnosis and treatment may be made through wireless communication with the doctor. In an emergency, if the doctor is in the air, help may literally be called down from the skies. The Far West Children's Health Scheme seeks out defects which may be or become crippling, but which are not severe enough to the lay eye to seem to warrant medical attention, or for which prolonged specialist treatment is not available except at prohibitive expense in large centres. The medical profession has helped Australia to put into effect a recognition of the rights of a dispersed population.

A country is not really grown up until it learns to welcome, to adapt itself to and to learn from the stranger, the foreigner, the migrant. In this Australia, through her history, has had limited opportunities. Our civilization has not been enriched by the foreign influences that have contributed to American and Canadian life. We have not had the opportunity of the frequent and easy acquaintance with foreigners in our own country or in theirs that is enjoyed by Europeans. There are lessons in human adaptation and tolerance, our learning of which isolation has hindered. We are apt to be impatient with the foreigner who is not perfectly familiar with our ways or master of our language, largely because we have not ourselves faced the task of making our way in a foreign country. The large migration programme since World War II promises to correct this imperfect understanding. Migrants are contributing graces and colour to our lives. Australians are learning the art and enjoying the satisfaction of trying to understand the stranger and of learning by helping him to understand.

A country means more than land, buildings, industry and a settled community life. It is also a tradition, a record of struggle, aspiration, generosity and smallness, achievement and frustration. This record is forever meaningful and sustaining, and a people does not truly understand itself until the feeling for history and tradition awakens and is cultivated. Our history is comparatively brief, and the oldest man-made objects in Australia (with the exception of the aboriginal) are recent compared with those of older civilizations. We give true depth to the study of our own history when we see it as a continuation of the history of Western Europe, and we must avoid the merely sentimental attitude that would confer on anything that is old an inflated claim to protection and preservation. But Australians at the moment need less a discouragement of this sentimental attitude than an exhortation to understand that we possess buildings and other works that, beside speaking eloquently of life in the past, have an enduring beauty and dignity of their own. We are still too heedless of them, and our heritage of them is not so ample that we can afford to see any of them destroyed or defaced or let go to ruin.

The time has come now when a resident of Sydney can read a life of Macquarie and of the convict architect Greenway and then walk round and see the remains of the noble plan that Macquarie began for the city.

Not the least of the signs of a country growing up is the cultivation of a feeling for history and tradition.

A. G. MITCHELL.

THE INCIDENCE AND DISTRIBUTION OF SCRUB TYPHUS IN NORTH QUEENSLAND[1]

E. H. DERRICK[2]

From the Queensland Institute of Medical Research, Brisbane

SUMMARY

In the four years 1951–1955, 199 cases or probable cases of scrub typhus were recognized in North Queensland. The geographical distribution was discontinuous along the Pacific Coast from near Thursday Island (10·6° S.), where cases have not previously been reported, to Mackay (21·1° S.). All but four occurred between Cooktown (15·5° S.) and Ingham (18·7° S.). Most cases arose in a narrow belt close to the sea, and all within 40 miles of it. Some arose on the Atherton Tableland at an elevation of 2000 to 3000 feet.

The scrub typhus region has an abundant rainfall and high humidity. Like the potential rain forest region, it is approximately bounded by the 60-inch isohyet. The mean annual temperature in the scrub typhus region ranges from 80·7° F. at Thursday Island to 68·1° F. at Atherton. The boundary of the region is not related to any isotherm.

The existence of foci of high and persisting endemicity, noted in other countries, is evident also in North Queensland. They offer opportunities for intensive ecological studies and for evaluation of preventive measures.

The highest monthly incidence was from March to July. The seasonal variation appears to be determined primarily by the rainfall—the wet season is from January to April—but modified by the occupational or recreational activities that take people into endemic foci.

Infection was associated with occupation in about 65%—mainly timber-cutting, scrub-clearing and fruit-farming—and in 18% with recreation. In 17% the significant activity was not defined.

SINCE the 1920's, scrub typhus has been recognized as one of the health problems of North Queensland. The aim of the present study is to define the magnitude of the problem and the localities at risk, and to consider some ætiological factors.

The data consist of three series of cases. Series I has been the most closely studied. Series II and III are needed for an estimate of the total incidence. Series III gives supplementary information about endemic localities, particularly those remote from Innisfail.

INCIDENCE

Series I

In July, 1951, a Field Station of this Institute was founded at Innisfail, North Queensland, for the intensive study of the endemic fevers of the region. Of these, scrub typhus has a high incidence, second only to leptospirosis. During the four years to June, 1955, the diagnosis of scrub typhus was established in 118 cases. Early in the period, the diagnosis depended on a characteristic clinical picture together with the detection of serum agglutinins to *Proteus* OX K. An eschar was also accepted as diagnostic in the absence of *Proteus* agglutination, as tick typhus, the only other North Queensland infection producing an eschar, is rare in the area from which the Field Station cases were drawn. From March, 1953, mouse inoculation was used systematically to investigate fevers, and this established the diagnosis of scrub typhus in cases without eschar or *Proteus* agglutination. The investigation of 32 of the cases by mouse inoculation was reported by Carley *et alii* (1955) and the clinical features of 53 by Doherty (1956).

Of the 118 cases, *Proteus* OX K agglutination was found in a titre of 1 : 80 or over in 63, an eschar was reported in 53, and *Rickettsia tsutsugamushi* was isolated in mice in 58. These gross figures may give an incorrect impression of the diagnostic significance of these criteria. In 76 cases in which the diagnosis of scrub typhus was established by eschar or mouse inoculation, and in which *Proteus* OX K agglutination was tested for between the eleventh and forty-second days, it was found

[1] Received on June 8, 1961.

[2] Deputy Director.

in a titre of 1 : 80 or over in 37, or 49%. In 94 cases in which the diagnosis was established by mouse inoculation or *Proteus* agglutination, an eschar was reported in 33, or 35%. This might be regarded as a minimum figure, as the proportion was higher in some groups that presumably received more intense clinical study. Blood was inoculated into mice in 42 cases in which the diagnosis was established by *Proteus* agglutination or eschar, and *R. tsutsugamushi* was isolated in 38, or 90%. Of the four failures, in one the blood was taken when the patient was afebrile after two days on chloramphenicol, in another on the sixteenth day of illness, in one contaminating organisms in it killed the mice, in the fourth no reason for the failure was evident.

TABLE I

The Incidence of Scrub Typhus in North Queensland, 1951–1955

Year	Series I Field Station Cases, Proved	Series II Field Station Cases, Probable	Series III Additional Cases	Total
1951–1952 ..	29	24	10	63
1952–1953 ..	31	13	16	60
1953–1954 ..	30	2	8	40
1954–1955 ..	28	3	5	36
Total ..	118	42	39	199

Series II

In addition to the 118 established cases, there were another 42 investigated at the Field Station in which the above-mentioned criteria were not met, but the clinical findings and history of exposure supported a probable diagnosis of scrub typhus. Most of these occurred in the period before mouse inoculation added precision to the diagnosis.

Series III

Series III was based on cases notified by practitioners as scrub typhus or suspected scrub typhus. Information about them was made available through the courtesy of Dr. A. Fryberg, Director-General of Health and Medical Services, and of Dr. (now Professor) D. Gordon, who investigated them. Among those occurring in 1951–1955 and not associated with the Field Station, a diagnosis of scrub typhus was acceptable in 39. In 25 the *Proteus* OX K agglutination titre was 1 : 80 or more, in five others an eschar was present, and in nine the diagnosis was based on circumstantial and clinical histories.

These three series give a total of 199 cases or probable cases of scrub typhus recognized in Queensland in the four years studied. The incidence in successive years (Table I) tended to decline.

During this period, 105 cases were notified as scrub typhus or suspected scrub typhus. The known incidence was, therefore, about twice the number of notifications. In the five years from 1955–1956 to 1959–1960, notified cases have numbered 14, 26, 22, 4, 9. The reason for the considerable reduction in the last two years, and whether or not it will be maintained, are matters for speculation.

SOME NOTEWORTHY CASES

There were two fatal cases in Series I, giving a fatality rate of 2%.

M.S., a pensioner, aged 68 years, had come from the Atherton Tableland five months before his illness to assist his son on his fruit farm at Mission Beach. He was ill about 17 days before his admission to Tully Hospital on March 7, 1952, and died two days later. " He had never been sick in his life before and thought he could fight it off."

J.P., aged 27 years, a timber-worker from Cape Tribulation, was ill for two weeks before his admission to Cairns Hospital on July 13, 1952. He died three days later. His late admission because of remoteness from hospital, and the long sea journey by launch, probably contributed to his death.

One man had a second attack, if one may judge by the agglutination results. Another instance, from Dr. Gordon's earlier records, may be cited.

F.R., aged 33 years, a fruit-farmer of Mission Beach, had his first attack in June, 1952. His titre of *Proteus* OX K agglutination rose from nil to 1 : 640. The second attack occurred in August, 1956. His serum, taken on the fifteenth day, agglutinated *Proteus* OX K to 1 : 1024.

C.L., aged 55 years, a cane-farmer of Fig Tree Pocket, 10 miles north of Babinda, was admitted to hospital with scrub typhus in July, 1949, and again in February, 1951. On each occasion he developed significant agglutination to *Proteus* OX K.

There were seven examples of multiple cases in a family.

A fruit-farmer at Mission Beach had scrub typhus in July, 1950 (before the present series), a daughter in April, 1952, his wife in June, 1952, and a son in April, 1955.

Two brothers were infected on their farm at Mission Beach in March, 1954, and April, 1955.

A mother and son were infected on their dairy farm at Daintree in May, 1953, and March, 1955.

Another mother and son were infected in September and November, 1954, after visiting scrub near their home at Flying Fish Point.

Two Cairns children, brothers, were both ill in December, 1952, and a Cairns husband and wife together in April, 1955.

A wife who visited her husband's timber camp at Bramston Beach developed scrub typhus in December, 1952, two months after he did.

Age and Sex

The age ranged from four to 72 years (Table II). The high proportion of males (81%) is explained by occupations that take men into forest and field.

Table II

Scrub Typhus: Age and Sex Distribution

Series	Male	Female	Total
Series I:			
0–4 years ..	0	1	1
5–9 years ..	3	3	6
10–14 years ..	4	1	5
15–19 years ..	8	2	10
20–29 years ..	29	4	33
30–39 years ..	22	6	28
40–49 years ..	10	3	13
50–59 years ..	14	2	16
60–69 years ..	5	—	5
70 years and over	1	—	1
Total	96	22	118
Series II	32	10	42
Series III	33	6	39
Grand total ..	161	38	199

Geographical Distribution

The localities where the Field Station cases arose (Table III, Figure I) lie in a narrow coastal strip extending from Cooktown (15·5° S.) to Ingham (18·7° S.), a distance of 230 miles.

Table III

Scrub Typhus: Geographical Distribution[1]

Site of Infection	Series I	Series II	Series III	Total
Thursday Island District	—	—	2	2
Cooktown District ..	6	1	3	10
Douglas Shire ..	15	7	2	24
Cairns City and environs	2	2	11	15
Mareeba Shire ..	1	—	—	1
Mulgrave Shire:				
Division 4	2	2	1	5
Division 1	12	5	1	18
Division 2	12	—	—	12
Division 3	20	3	1	24
Johnstone Shire:				
North Mission Beach	19	7	2	28
Rest of Shire ..	17	7	—	24
Cardwell Shire ..	3	2	1	6
Hinchinbrook Shire ..	3	—	5	8
Atherton Tableland:				
Atherton Shire ..	—	—	2	2
Eacham Shire ..	3	2	3	8
Herberton Shire ..	—	2	3	5
Mackay District ..	—	—	2	2
Locality of origin undefined	3	2	—	5
Total ..	118	42	39	199

[1] A table defining sites of infection in detail has been filed at the Queensland Institute of Medical Research.

This is essentially the same distribution as with the combined series of Mathew (1938) and Heaslip (1941), although the local emphasis has varied over the years according to the site of such activities as road-making and scrub-clearing. A general description of the area

Figure I

Map of the main scrub typhus region. X indicates places of origin of representative cases in the present series. With one exception, they lie on the wetter side of the 60-inch isohyet. Shires are indicated as follows: A, Atherton; C, Cook; CL, Cardwell; D, Douglas; E, Eacham; H, Herberton; HK, Hinchinbrook; J, Johnstone; M, Mareeba; 1, 2, 3, 4, Divisions of Melgrave

TABLE IV

Some Locality Records of Scrub Typhus

Patient	Sex	Age (Years)	Locality	Approximate Altitude (Feet)	Occupation	Date of Illness	Reciprocal of Proteus OX K Titre	Remarks
				Thursday Island District				
J.T.	M.	42	Cowal Creek	—	Teacher	August, 1951	640	Temperature 104° F. on admission, headache, joint pains, conjunctival injection
K.S.	F.	20	Hammond Island	—	Domestic duties	February, 1954	256	Temperature 103° F., severe headache, rash, adenitis, conjunctivitis
				Atherton Tableland				
E.T.	M.	28	Rocky Creek	2200	Maize-farm labourer	December, 1952	1000	Eschar
C.N.	M.	28	Minbun	2500	Timber-cutter	May, 1952	Nil	Rash, probable eschar
W.C.	M.	41	Seven miles south of Millaa Millaa	2700	Timber-cutter	April, 1953	80	
C.J.	M.	46	Tully Falls (upper level)—Ravenshoe	2300–3000	Surveyor's labourer	August, 1953	125	
W.C.	M.	32	Tully Falls (upper level)	2300	Labourer	April, 1952	160	
				Mackay District				
B.M.[1]	M.	39	Balnagowan	—	Cane-farmer	March, 1953	640	Had been cutting guinea grass
C.W.	M.	24	Silent Grove	—	Truck-driver	December. 1954	128	Trucked cane from farms to railway

[1] This case was also included in the report by Derrick *et alii* (1953)

has been given by Derrick *et alii* (1954). The wider distribution of Series III draws attention particularly to the Thursday Island, Atherton and Mackay districts. All the cases here reported arose within 40 miles of the sea, 80% of them within 10 miles.

Thursday Island District

The two cases in Series III (Table IV) provide the first evidence of scrub typhus in this area. Both patients were admitted to Thursday Island Hospital severely ill with fever, and developed agglutinins to *Proteus* OX K. There is no information about the circumstances which led to their infection. Cowal Creek, where J.T., a Badu Islander, was employed as a teacher, is an aboriginal settlement on the mainland of Cape York. The country there is mostly open forest, with rain forest along creeks or on pockets of good soil. Hammond Island, adjacent to Thursday Island on the west, is lightly timbered and has much long grass, but no rain forest.

Dr. J. I. Tonge has informed me of two further patients with scrub typhus who were admitted to Thursday Island Hospital in 1956.

D.P., a male patient, aged 16 years, had been cutting grass in a swampy area at Cowal Creek. He became ill on July 31 and failed to respond to penicillin. He showed a rash and lymphadenitis, and his serum agglutinated *Proteus* OX K to 1 : 2048.

N.T., a diver on a lugger working near Darnley Island, became ill on August 2, and was admitted to hospital on August 8, severely ill with a temperature of 104° F. The titre of *Proteus* OX K agglutination rose from nil to 1 : 512.

Cooktown District

The four timber-cutters, four tin-miners and one road-worker came from Rossville, Mt. Poverty, Bloomfield River and Cape Tribulation. One man had gone to work a tin mine near Mt. Poverty on the advice of an old prospector, who had vacated it 16 years earlier when he himself allegedly caught scrub typhus. An aboriginal boy lived on the Reserve at Cooktown.

Douglas Shire

Douglas Shire had the highest relative incidence—9·2‰, in a population of 2600—and also the highest proportion of females—eight of 24. Six of the females were housewives, one was a tourist, and one was a schoolgirl. Cases arose in many parts of the shire. They included four on two adjacent farms at Daintree and four, perhaps five, in a small area at Mowbray.

Cairns

Cairns is unique among Queensland cities, in that foci of scrub typhus exist within the city boundaries. Heaslip (1941) found the swamps of West Cairns a prolific source of human cases, naturally infected animals and *Trombicula deliensis*. The strong representation of Cairns in Series III is largely due to the diagnostic facilities offered by the Commonwealth Health Laboratory there. The homes of six Cairns residents were beside thick jungle, which was the probable site of infection. Two were employed by the Anti-Malaria Unit of the

Cairns City Council for spraying swamps. The site of infection of the other seven was not defined; some may have been infected outside the city boundaries in Mulgrave Shire. In recent years, the rapid expansion of suburban Cairns has led to elimination of much jungle and swamp and a consequent lessening of the scrub typhus risk.

Mulgrave Shire

During the war, many cases of scrub typhus arose among soldiers camping or exercising in this shire. Localities concerned included Dead Man's Gully (the site of "Outbreak B" of Cook, 1944), Green Hill (Gordon, 1948) and the hills behind Hambledon (personal communications from J. P. O'Shea and Australian War Memorial). Cases in the present series have been scattered throughout the shire from Yorkey's Knob to Waugh, with a special concentration at Yarrabah in Division 1 and Bramston Beach in Division 3. The list includes three from near Green Hill, four from Little Mulgrave and three from Meerawa. In December, 1951, three persons were infected within ten days at a focus south of the Russell River—a farmer clearing new land for cane, a man assisting him, and a housewife on an adjoining canefarm.

Yarrabah is an aboriginal settlement with a population of about 700. The terrain includes hills and swamps and is largely covered with rain forest where it is not cleared for settlement. Adult males are employed on the station in maize, fruit and vegetable farming, dairying and saw-milling. The nine patients from Yarrabah were full or part aborigines. These are, therefore, not racially immune to scrub typhus.

Bramston Beach is an attractive beach five miles long opposite Babinda. A road leads in near its southern end, and here a township has been surveyed. The township site lies on a sandy strip about 100 yards wide. Inland to this is a swamp of the same width. The flora, in turn from the sea, consists of (*a*) low herbage growing in the sand (Figure II), (*b*) beach forest of bewildering variety dominated by *Casuarina* and including also *Eucalyptus tesselaris*, large clumps of *Scævola frutescens* and acacias (Figure III), which merges into (*c*) swamp forest. Grasses, of which blady grass (*Imperata cylindrica* var. *major*) is the commonest, grow sparsely under the trees and freely in clearings and beside the roads. North of the township the swamp widens to over a mile. A forestry road to the south leads past an old coconut plantation. Nearby hills are clothed with dense rain forest. (For points in this description, the writer is indebted to Dr. M. J. Mackerras and Dr. J. L. Harrison.)

Scrub typhus first came to notice at Bramston Beach during construction of the road in 1941. Notoriety followed an outbreak of over 70 cases among 3000 troops who exercised there in February, 1944 (Southcott, 1947).

FIGURE II

Bramston Beach, 1953. On the left is shown the zone of low herbage nearest the sea

After the war it became a popular spot to visit or camp in on holidays, and further cases followed. In 1947 there were five cases, one fatal (Gordon, 1948). In November, 1948, 26 allotments in the township were purchased, but two further cases, one in a man clearing his

FIGURE III

Bramston Beach, 1953. Beach forest of varied composition, further inland. Grass, mostly *Imperata cylindrica*, beside roadway

allotment, discouraged hut-builders. Mulgrave Shire Council gave warning of the typhus risk by newspaper advertisements and notices at the beach—"Scrub Typhus Area. Camping Prohibited." For a few years visitors were few. The Council also supplied dibutyl phthalate

free to anyone throughout the shire who asked for it. Council employees used it on their clothing.

In 1953, the permanent population consisted of three men. In that year, a women's organization arranged to have a hut built at the Beach for recreational convenience (Figure IV). A member visited it on April 19, but feeling ill with a sore throat remained in the partly-built hut until she was driven out by the noise of construction. She then lay on a rug on the grass nearby. On April 27, the onset of scrub typhus appeared in the form of a throbbing eschar in the axilla. The other ten patients in

FIGURE IV

Recreation hut at Bramston Beach, 1953. A woman developed scrub typhus after lying on a rug on the grass in the foreground. On the right, a trunk of *Eucalyptus tesselaris* is recognizable by the persisting dark bark around the lower part

the present series infected at Bramston Beach included five men who were camped there while they cut timber and firewood; the wife of one of these and the wife of an unaffected timber-cutter who had recently visited their husbands' camps; and three other men who had visited the Beach at week-ends to collect timber. The main timber-cutting area was several miles north of the township.

In recent years, Bramston Beach has again become popular and the site of large picnics. At least 20 houses, mostly temporary, have been built, and at present scrub typhus does not appear to be a problem in the township area.

Johnstone Shire

Johnstone Shire contains numerous endemic foci.

Flying Fish Point (population 232) is situated four miles from Innisfail at the mouth of the Johnstone River and is backed by a hill clothed with rain forest. Doherty (1957) observed that sides of roads and vacant areas were used as rubbish dumps. From August to November, 1954, four people were infected here—a housewife, her son aged five years, a high-school student and a carpenter.

Seven men were infected while cutting timber or firewood or clearing scrub on a group of adjacent properties near Mourilyan Harbour, also a housewife living nearby. This area has provided cases at intervals since 1938; in that year two timber-cutters from Mourilyan Swamp were included in Heaslip's series. M. J. Mackerras and J. H. Pope isolated *R. tsutsugamushi* from a rat, *Melomys cervinipes* (Gould), captured in July, 1957, at a place where a firewood-cutter was infected in April, 1955; the trap was set just within rain forest close to the road.

An intense focus exists at Mission Beach. This beach, eight miles long, takes its name from an Aboriginal mission or settlement, which was established there in 1914 by the Queensland Government, but destroyed on March 10, 1918, by a cyclone, which also killed the superintendent, J. M. Kenny, and his daughter. Near Tam O'Shanter Point, at its southern end, E. B. Kennedy landed in 1948 to begin his exploration to Cape York. Timber-getting was the first industry. Later came fruit-growing, especially of bananas and pineapples. Cases of fever resembling scrub typhus have arisen near the beach for many years, as was emphasized in 1935 by Unwin. He referred particularly to an outbreak, with one fatal case, among workers building the road from El Arish to Clump Point.

About 1950, an area at the northern end of the beach was divided into small blocks, and the new occupiers began the heavy task of clearing the scrub to plant bananas and pineapples. Close to the sea, the flora is similar to that at Bramston Beach; a short distance inland it is dense rain forest (Figures V, VI, VII). Bringing the North Mission Beach scrub into production has provided 28 cases of scrub typhus in the present series. From February to July, 1952, there were 10 cases, one fatal, and these caused concern among the small population of 60. In August, the Johnstone Shire Council provided them with dibutyl phthalate for impregnation of clothing. It is not clear how much the incidence was reduced by this. There were five cases in 1953 and seven in 1954.

The Barnard Islands are uninhabited, but are much visited by fishing and shooting parties. In 1874, Dalrymple described them and included a report on the flora by Walter Hill, Queensland's

first Colonial Botanist. Dalrymple found one of the South Barnards a good place to camp—good water, wood, oysters, fish, pigeons and scrub hens. One man in his party, Trooper Billy, who camped there from November 2 to 10,

FIGURE V

Rain forest near Clump Point, showing dense regrowth along the margin, May, 1955. Photograph, R. L. Doherty

1873, was reported on November 17 to be suffering from the effects of severe attacks of fever. No details are given by which the fever can be identified. (Hill also had a fever—too short for scrub typhus—while in the Johnstone River area, and Dalrymple a prolonged fever—its place of origin undefined—after his return to Brisbane.) In the year 1947–1948 there were eight cases of scrub typhus among visitors to the North Barnards (Gordon, 1948),

FIGURE VI

A farmer at Mission Beach spraying his pineapples, May, 1955. He had scrub typhus in March, 1954, and his brother, on the same farm, in April, 1955. Dense rain forest in background. Photograph, R. L. Doherty

but the group is not represented in the present series. The Barnard Islands offer a possible site for an ecological study of scrub typhus; on an island the study area is circumscribed and isolated, the number of arthropod and mammalian species restricted and the epidemiological picture simplified.

Cardwell Shire

The six cases from Cardwell Shire are thought not to represent the full incidence. One man became ill in April, 1954, while clearing scrub on a pineapple farm at South Mission Beach. Eleven months earlier, his employer had been admitted to hospital in Geelong, Victoria, for scrub typhus contracted while inspecting his block; he had an eschar in the axilla.

FIGURE VII

Another fruit farm at Mission Beach, May, 1955. The boy, ill a month previously, was the fourth to contract scrub typhus on this farm in five years. Free growth of grass and weeds among the bananas. Photograph, R. L. Doherty

Hinchinbrook Shire

Abergowrie, where three timber-cutters, two cane-farm workers and a surveyor's labourer were infected, was opened up for sugar-growing in 1951. It is covered largely with open hardwood forest, with some rain forest along creeks.

Atherton Tableland

Much of the Tableland has been cleared for agriculture, dairying and cattle-raising. Much rain forest remains. Scrub-typhus foci are scattered from Rocky Creek (Southcott, 1947) to the Tully Falls.

Mathew (1938) reported a case each from Tolga and Atherton. A number of foci discovered during the war have been reported by C. E. Cook (1944) and Southcott (1947), and

through the courtesy of these authors, I. J. Wood, I. Cook, G. A. Jackman and the Australian War Memorial, some further detail may be added. "Outbreak A" of C. E. Cook, affecting 45 soldiers, arose at Severin Creek (altitude 2300 feet) four miles east-north-east of Kairi. In the four days January 11 to 14, 1944, two nurses and two machine-gunners from 2/2 Australian General Hospital developed scrub typhus after visiting, on Sunday, January 2, an area at Rocky Creek (altitude 2100 feet) used by hospital staff and convalescent patients for picnics and swimming. The area was thereupon declared out of bounds. Two cases in February, 1943, among female staff of the same hospital had possibly arisen at the same place. Southcott described a case arising in March, 1944, at a place (altitude 3000 feet) between Wondecla and Minbun; this was the only one in this area in spite of extensive troop training.

In the present series, there were 15 cases in the Atherton, Eacham and Herberton shires. The relative incidence on the Tableland is much lower than in the coastal belt. The Goldsborough logging area, where three timber-workers were infected, is at a low altitude on the eastern slope of the Tableland. A maize farm within two miles of the Rocky Creek picnic area mentioned above was the site of infection of a farm worker in December, 1952, as well as of his uncle in April, 1950. There are pockets of rain forest nearby. Some other localities are listed in Table IV.

The Atherton Tableland cases have several points of interest. They mark the western limit of this part of the scrub-typhus region. They show that cases can arise at an elevation of up to 3000 feet.

Mackay District

The Mackay District is the most southern locality in the world from which scrub typhus has been reported (Derrick *et alii*, 1953). It was first described here as "Sarina fever" (Wheatland, 1924). The two cases in the present series (Table IV) came from Balnagowan, 10 miles west, and Silent Grove, 27 miles west-north-west, of Mackay.

Northern Territory

This is the only region in Australia outside Queensland from which scrub typhus has been reported. In 1939, Kirkland noted that six cases of this disease had been reported in the Northern Territory, the first in 1937. In a personal communication (1951), he gave further information:

> The first case was in a boy, who was aged about 12 years and a resident of Darwin, but who went on frequent trips to the scrub and minor jungle country near Darwin. His clinical condition was in accord with scrub typhus as seen elsewhere, and agglutination of *Proteus* OX K was in quite high titre. The diagnosis of the other five cases was not so well attested.

Dr. J. M. Crotty, Commonwealth Health Laboratory, Darwin, informs me that he knows of only one case of scrub typhus in the Northern Territory of recent years:

> A male full-blood aboriginal, aged about 40 years, from Caledon Bay and Rose River, became ill on June 19, 1957, and was admitted to Darwin Hospital on June 26. His temperature was chiefly in the range 99° to 102° F., but occasionally rose to 104° F. The spleen was just palpable. No other abnormality on clinical examination was reported. The *Proteus* OX K titre rose from 1 : 1600 on July 5 to 1 : 20,000 on July 26. Investigations for malaria, tuberculosis, murine typhus, typhoid and urinary infection gave negative results. He was treated with chloramphenicol and made an uncomplicated recovery.

TABLE V

Analysis of 58 Strains of Rickettsia Tsutsugamushi According to Locality of Origin and Virulence for Mice[1]

Locality of Origin	Virulence			Total
	High	Intermediate	Low	
Cooktown District ..	1	—	2	3
Douglas Shire ..	4	—	3	7
Mareeba Shire ..	—	—	1	1
Mulgrave Shire:				
Yarrabah	3	—	2	5
Division 1, other ..	—	1	1	2
Division 2	—	2	4	6
Bramston Beach ..	—	2	5	7
Division 3, other ..	4	—	1	5
Johnstone Shire:				
Flying Fish Point ..	2	—	2	4
Mourilyan Harbour	2	—	2	4
Mission Beach ..	4[2]	—	2	6[2]
Other	1	1	1	3
Hinchinbrook Shire ..	1	1	—	2
Eacham Shire ..	—	—	1	1
Undefined	1	—	1	2
Total —	23	7	28	58

[1] The table includes the 31 strains reported by Carley *et alii* (1955). These authors noted significant correlation between virulence for man and for mice.

[2] Including one strain that originated in South Mission Beach, in Cardwell Shire.

Geographical Distribution and Virulence

Carley *et alii* (1955) found that there was little or no correlation between virulence of *R. tsutsugamushi* and locality of origin. This is confirmed by analysis of the 27 further strains isolated from patients by the same workers. In most foci the numbers of strains of high and low virulence were about the same (Table V). However, no highly virulent strains were among the few isolated in Division 2 of Mulgrave Shire or at Bramston Beach. The low mortality

(one death in over 70 cases) in the 1944 outbreak at Bramston Beach suggests that most, if not all, of the infecting strains on that occasion were of low virulence. The strain isolated from *M. cervinipes* at Mourilyan Harbour was highly virulent.

OCCUPATION AND ACTIVITY

In Table VI, 118 patients are classified according to the activities that led them into or near endemic foci.

TABLE VI

Scrub Typhus: Activities Prior to Illness

Activities	Number of Patients[1]
Occupational activities:	
Cutting timber or firewood	24
Clearing scrub (including rain forest)	13
Fruit-farming at Mission Beach	15 (1)
Fruit-farming and surveying	1
Fruit-farming and timber-cutting	1
Cane-farming	10
Mixed farming and dairy-farming	3
Alluvial tin-mining	3
Various work in or near scrub:	
On roads	3
On bridge, forestry, at sawmill, repairing house	4
Recreational activities	21 (7)
Relevant activity unknown:	
Housewives 9 (9); children 8 (5); teacher; others 2	20 (14)
Total	118 (22)

[1] Figures in parentheses indicate number of females.

In 77 cases (65%), infection was associated with occupation. Among males aged 15 years and over, this proportion rose to 84%. With only one of the females could infection be definitely ascribed to occupation; she assisted on a fruit farm at Mission Beach.

The commonest occupational activity was timber-cutting. This and scrub-clearing entailed prolonged contact with a potentially infected environment. Three of the 24 timber cutters had other regular occupations; they were infected while getting timber for personal use during a week-end. Eleven of the scrub-clearers were preparing for the planting of sugar cane. The 15 fruit-farmers or farm-workers at Mission Beach lived and worked in a highly infective area; the work of six had included scrub-clearing. Fourteen men worked on cane-farms, apart from the 11 scrub-clearers mentioned above. Sugar fields themselves probably carry no risk of scrub typhus, and four of the cane-farm workers, who gave a definite history of shooting, pig-hunting, etc., have been included in the recreational group. In default of such a history, infection of the other 10 has been regarded as probably occupational, although its occurrence during week-end recreation cannot be excluded. The work of some of these men is known to have taken them into uncleared areas on the farm.

Twenty-one patients, or 18%, had entered rain forest for recreational reasons—touring, picnicking, camping, pig-hunting, shooting, collecting ferns or orchids.

In 20 cases, or 17%, the relevant activity was not recorded or not clearly defined. Among these, two children and a teacher lived at Mission Beach. Three of the housewives lived on cane farms, two on dairy farms; two had visited their husbands' timber-cutting camp at Bramston Beach and one her husband's grazing lease.

SEASON

Cases occurred in every month, with a marked predominance from March to July (Figure VIII).

The most important factor in producing this seasonal distribution appears to be rainfall. In coastal North Queensland, the wet season usually extends from January to April. At Innisfail, for instance, 88 inches of the annual mean total of 139 falls during these four months. The main typhus season thus corresponds with the latter part of the wet season and the subsequent three months.

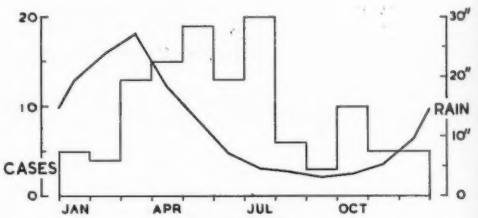

FIGURE VIII

Scrub typhus in relation to rainfall. The histogram shows the monthly distribution of 118 cases, the continuous line the mean monthly rainfall at Innisfail

Of previous North Queensland series, only that of Mathew (1938) is adequate for seasonal analysis; it does not show a relation to the wet season; the months of highest incidence were June and July in 1935 and September in 1936. Heaslip's (1941) cases were discontinuous; among the months represented, April, 1939, and May, 1940, were the most prolific. The Severin Creek outbreak of Cook (1944) was in May and June, 1943, that at Dead Man's Gully from July 2 to 20, 1943, and that at Bramston Beach (Southcott, 1947) in February, 1944. Wheatland (1924) noted that cases of "Sarina fever" occurred from November to April.

In monsoonal regions in Asia, an association between scrub typhus and the wet season has been widely reported. In Burma, Sayers (1946)

noted an overwhelming preponderance of cases in the monsoon and post-monsoon seasons. In Calcutta, Krishnan *et alii* (1949) found the maximum prevalence during the rainy season from May to October. In Canton, Hsu *et alii* (1959) noted that cases of scrub typhus appeared most abundantly after a month or so of heavy rain. The seasonal upsurge of typhus was observed by Audy and Harrison (1951) at Imphal and by Krishnan *et alii* (1949) at Calcutta to coincide with the striking increase of larval *T. deliensis* that followed the first heavy rains. The number of these mites in turn fell rapidly with the onset of dry weather, but some persisted in moist foci throughout the dry season.

While the seasonal distribution of *T. deliensis* is thus closely related to the rainfall, that of scrub typhus is also dependent on the activities that bring people into mite-infested foci. In North Queensland, as Mathew noted in 1938, more men are employed on farms, on road-making, on scrub-clearing and on timber-getting during the cooler and drier months. In the wet season, the hauling out of timber becomes impracticable and the jungle less attractive to visitors.

TEMPERATURE

The mean annual temperature in the scrub typhus region varies from 80·7° F. at Thursday Island to 68·1° F. at Atherton (Table VII). A similar range of temperature is found in neighbouring places without scrub typhus. It is not decreasing temperature that sets the southern limit of endemicity near Mackay, as the temperature along the coast exceeds that at Atherton as far south as Brisbane.

RAINFALL, RAIN FOREST AND HUMIDITY

The known foci of scrub typhus in North Queensland possess an abundant rainfall, which usually exceeds an annual mean of 60 inches, and rises to 175 inches at Tully. That is, the potential scrub typhus region is approximately delineated by the 60-inch isohyet. This cuts across the top of Cape York Peninsula, reenters west of Cape Melville, and leaves again south of Ingham. Another section of it encircles Mackay (Figure IX). Scrub typhus is not continuously distributed throughout the regions of high rainfall, but is restricted to scattered foci.

Herbert (1960) notes that, with some reservations, the 60-inch isohyet forms the approximate boundary of rain forest. Rain forest requires also that the rainfall reaches a certain minimum in the drier months, and that the soil is suitable. Attenuated rain forest may extend along streams into drier regions. There is thus a general correspondence between potential scrub typhus and rain forest regions. As rain forest implies sustained as well as high humidity, it may be a better indicator than annual rainfall, which may be mostly in one season, that the high humidity requisite for *T. deliensis* is present.

Table VII shows that the relative humidity is consistently high where scrub typhus occurs, and as a rule higher than in neighbouring places where it is not present.

FIGURE IX

Rainfall map of Queensland. A series of coastal regions, in black, receive an average of at least 60 inches annually. Reports of scrub typhus have been confined to the most northerly three of these

As was noted above, the history of many patients in the present series pointed to rain forest as the source of their infection. Some worked on its fringe (Figures VI, VII). With some, the site of infection was definitely not rain forest, as in Bramston Beach township (Figure IV). From his experience in Asia, Audy (1949) concluded that scrub typhus is apparently not native to virgin rain forest. All definitely pin-pointed infections in the Burma campaign occurred in connexion with "scrub"—that is, the mixed vegetation that springs up in an area that has been cleared and then neglected. (To avoid confusion it should be explained that in Queensland virgin rain forest is called "scrub".) In New Guinea, scrub typhus was notoriously associated with kunai grass (*I. cylindrica* var. *major*) and with overgrown, abandoned gardens. With the Queensland

patients who worked or sported in rain forest, it cannot be excluded that some were infected as they necessarily traversed the fringe zone. Evidence of the relative infectivity of rain forest, secondary growth, fringe and grass in Queensland must await surveys of the distribution of *T. deliensis* and its mammalian hosts.

No cases of scrub typhus have yet been recognized between Cowal Creek and Cooktown, but the possibility exists of some foci in this very sparsely populated area. Herbert notes that there are three extensive areas of true rain forest in the Peninsula—near Cape York, in the hinterland of Weymouth Bay and Lloyd Bay, and on the eastern slopes of the McIlwraith Range. The rainfall is approximately 58 inches at Lockhart River and exceeds 60 inches in a strip from Cape Melville to Cooktown (Figure IX).

TABLE VII

Rainfall, Humidity and Temperature at Representative Localities[1]

Locality	Mean Annual Rainfall (Inches)	Mean Annual Relative Humidity (Percentage)	Mean Annual Temperature (Degrees Fahrenheit)
Scrub typhus recorded:			
Thursday Island ..	66·4	79	80·7
Cooktown ..	67·9	77	78·1
Cairns	86·4	76	76·3
Innisfail	139·2	84	73·9
Ingham	80·6	72[3]	75[3]
Atherton	54·0[2]	77	68·1
Mackay	63·2	79	72·2
Scrub typhus not recorded:			
Normanton ..	37·6	54	80·9
Townsville ..	43·1	70	76·0
Bowen	36·4	73	75·1
St. Lawrence ..	37·2	71	72·5
Nambour	65·1	73[3]	67[3]
Brisbane	40·1	68	69·0

[1] From "Climatic Averages, Australia", Commonwealth of Australia, Bureau of Meteorology (1956), and Brisbane Weather Bureau.
[2] The 60-inch isohyet lies just to the east of Atherton.
[3] Approximate.

Although no case of scrub typhus has been known to arise in southern Queensland, some coastal areas there, as for instance around Nambour, appear to be climatically suitable. In them, the rainfall exceeds 60 inches (Figure IX), rain forest abounds, and the temperature is as high or almost as high as at Atherton (Table VII). It is not likely that the Atherton temperature is a limiting one, as scrub typhus occurs in northern Japan and the Himalayas, where winters are snowy. Some coastal places in New South Wales, therefore, such as Byron Bay and Coff's Harbour, where the rainfall exceeds 60 inches, may also be climatically suitable.

It should be noted that Audy (1949) has reported the occurrence of scrub typhus in localities in Central Burma with an annual rainfall of less than 40 inches.

CONCLUSION

This study raises some questions and opens up fields for further inquiry.

1. Can the decline in incidence of scrub typhus in recent years be accelerated by a wider use of impregnated clothing or by disinfestation by miticides of selected localities? Human cooperation is, of course, as important as technical efficiency.

2. What is the distribution of *T. deliensis* and other trombiculid mites in relation to the distribution of scrub typhus? *T. deliensis* has so far been reported from only a few localities in North Queensland—Cairns (Heaslip, 1941), Bramston Beach (Mackerras *et alii*, 1949), Flying Fish Point (Domrow, 1955) and Innisfail (Domrow, 1956).

3. An intensive ecological study at some selected foci, such as Mission Beach or the Barnard Islands, would provide valuable information on mites and their hosts, and on the conditions that favour *R. tsutsugamushi*.

ACKNOWLEDGEMENTS

The writer is indebted to many people (some of whom have been acknowledged in the text) for information used in this study, particularly Dr. R. L. Doherty, Dr. C. N. Sinnamon, Professor D. Gordon, Dr. J. I. Tonge, Dr. R. V. Southcott, Dr. V. E. Knight, Dr. P. B. Wallis, Inspector J. M. Kennedy, Inspector D. Kennedy, Inspector J. P. O'Shea, and the Director of the Australian War Memorial.

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CONGENITAL MALFORMATIONS AND MATERNAL DIET[1, 4]

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SUMMARY

Nutritional deficiency has been shown by many workers to be teratogenic to the animal embryo. Vitamins of the B group especially appear to be essential for normal organogenesis. This is consistent with the known general functions of the B vitamins, which, acting as intracellular enzymes, promote rapid cell growth, for example, in the adult hæmatopoietic and gastro-intestinal systems.

In this study an attempt has been made to detect deficiencies in the diets of 99 mothers who bore malformed children. When their estimated diets were compared with those of a matched control series, some small differences were found in several groups of malformation, especially congenital heart disease and talipes. The most usual differences were in iron and the B group vitamins, thiamine and niacin; but whether these differences are biologically important cannot be stated. No differences were found in mongolism and spina bifida.

THE pathology of the unborn child is a field of human biology that is of definite interest to the physician, for it is in this field that there operate those little-known factors which result in the birth of a defective individual. Usually termed "congenital" as opposed to "acquired", these defects, which are present at birth, ought to be defined more exactly. We should be familiar with the terms "embryopathy" and "fœtopathy", the former relating to events occurring during the organogenetic period of early pregnancy, the latter referring to diseases of the fœtus, such as syphilis and toxoplasmosis. Hereditary conditions or phenotypes are, of course, determined earlier, at conception.

Congenital malformations may therefore be spoken of as phenotypes, embryopathies and fœtopathies. Their causes may be represented as a spectrum (Figure I) ranging from the environmental embryopathies such as irradiation and rubella, to the known phenotypes. These two groups probably account for only a minority of malformations, there being in between a large *terra incognita* where unknown causes operate. It is this field which we propose now to discuss, and there it is suspected that both environmental and hereditary factors are operating, either singly or in combination.

This combination of causes, or double ætiology, is a phenomenon well known to the animal experimenter, from whom we have gained much collateral evidence in this field. Some of this evidence is summarized in Table I, in which we see that there are various "insults" which may experimentally produce malformations in the animal embryo. Anoxia, cortisone, irradiation and other stimuli applied to the mother animal will regularly produce malformed offspring, as will also dietary deficiencies of various vitamins. In much of this work—for example, that of Nelson *et alii* (1952), Kalten (1959) and Pinsky and Fraser (1959), the dietary deficiency was complete, a state of virtual avitaminosis being produced by the use of antivitamins.

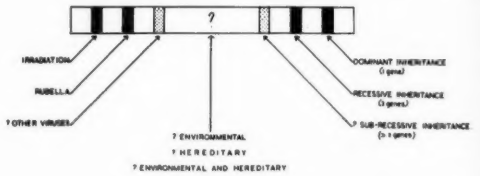

FIGURE I
Spectrum of human malformations

There are three features in all this work that deserve comment. (i) The susceptibility of the embryo to noxious influences often varies from strain to strain, owing to differing genetic constitution. (ii) The teratogenic effects are often non-specific for the particular stimulus; for instance, cleft palate has been shown to be caused by several of the abovementioned factors. (iii) Time-specificity is often operative—that is, the nature of the defect can be varied by altering the embryonic age at which the stimulus

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TABLE I

Some Teratogenic Factors (Animal)

Animal	Factor	Effect	Reference
Sow	Hypovitaminosis A	Anophthalmia, microphthalmia	Hale (1935)
Rat	Hypovitaminosis A	Ocular defects. Mesodermal defects in genito-urinary and cardio-vascular systems	Wilson and Warkany (1949)
Rabbit	Hypovitaminosis A	Hydrocephalus	Millen *et alii* (1953)
Rat	Ariboflavinosis	Skeletal malformations, cleft palate	Warkany (1944)
Mouse	Ariboflavinosis	Skeletal defects, œsophageal dysplasia, cerebral defects and hydrocephalus. (N.B., inter-strain differences)	Kalter (1959)
Rat	Folic acid deficiency	Cleft palate, syndactyly, cataract, hydronephrosis, etc.	Nelson *et alii* (1952)
Mouse	Niacin deficiency	Skeletal defects, cleft palate	Pinsky and Fraser (1959)
Mouse	Anoxia	Anencephaly, fusion of ribs, hemivertebra, cleft palate, etc.	Ingalls *et alii* (1952)

is applied. This agrees with the known ontogenetic data of development, and with the clinically observed time-specificity in rubella embryopathy.

The classical studies of Hale, Warkany and others have thus shown that maternal vitamin deficiencies, particularly of the B group, are teratogenic to the animal embryo and produce a wide variety of congenital malformations. Such observations have, of course, posed the question whether nutritional deficiency is teratogenic in man? Surprisingly little work has been done previously on this important question, and the only controlled study that we are aware of is that of Burke *et alii* (1943), who conducted at the Boston Lying-In Hospital a nutritional prospective study on pregnant women and their babies. The diets of these women were tabulated by means of food

TABLE II

Adapted from Burke *et alii* (1943)

Result	"Good" or "Excellent" Diet	"Poor" or "Very Poor" Diet	
Normal infant ..	27	11	38
Congenital defect ..	1	9	10
Total ..	28	20	48

$\chi_1^2 = 9{\cdot}76^{**}$
$P < 0{\cdot}01$.

histories, and graded by a loose five-point rating system, which compared the intake of nutrients against the prevalent recommended optimal allowances. The results of these workers are summarized in Table II, which shows that a significant excess of malformed infants were born to mothers who were assessed as having eaten poor diets.

This investigation suffers from the disadvantage of being tied to an arbitrary scale of recommended food allowances. These scales vary from time to time and from country to country, and, since a liberal margin of the various nutriments is allowed, tend therefore to cause bias.

TABLE III

Malformations Studied

Condition	Number of Cases
Mongolism	8
Spina bifida cystica	13
Cleft lip±cleft palate	17
Congenital heart defects[1]	11
Talipes	20
Other musculo-skeletal defects[2]	16
Miscellaneous defects[3]	14
Total	99

[1] Fallot's tetrad (3), ventricular septal defect (2), transposition of great vessels (2), truncus communis arteriosus (2), pulmonary stenosis, pulmonary stenosis and auricular septal defect.
[2] Hypophalangy, syndactyly, hemimelia (3), achondroplasia (2), herniæ (2), polydactyly (5), fibroma, lipoma.
[3] Hirschsprung's disease, micrognathia, tracheo-œsophageal fistula, epispadias, adreno-genital syndrome, multiple malformations, intestinal atresia (2), microcephaly, hydrocephalus (2), hydrocephaly, hypospadias (2).

PRESENT STUDY

The present investigation sought to overcome this difficulty by ignoring the recommended allowances and using controls instead.

In the course of a three-year investigation of malformed infants born at the Royal Women's Hospital, Melbourne, 99 of their mothers were available for interview by us, their choice being governed by random factors. A food history was taken, which sought to establish the usual dietary pattern of each mother. This was subsequently analysed into the various dietary constituents according to the "Tables of Composition of Australian Foods" (Osmond and Wilson, 1954). These mothers bore children with the malformations shown in Table III. In addition, 99 mothers of normal infants were

chosen by one of us (B.D.P.) as controls, the criteria for matching being maternal age, parity, ethnic grouping (British, Northern European, Southern European, and other) and sex of child. In other respects these patients were randomly chosen. The first three were obviously important standards for matching, as dietary habits vary within each group. These patients were then interviewed, and food histories were taken by the dietitian working " blind " ; that is, she was not told which was an abnormal case, so as not to be biased in her assessment.

Type of History Required for Research

The four main methods for collection of data of food consumption have been discussed by Trulson and McCann (1958). These methods are : (i) dietary record or diary ; (ii) weighing of food consumed ; (iii) questionnaire ; (iv) dietary history interview.

As the present study was done in retrospect, the most suitable method of collecting information was considered to be the dietary interview.

In the paper published by Trulson (1954) assessing dietary study methods, the following conclusions were reached. Dietary data obtained from three methods—seven-day record, dietary interview of usual food practices, and three or more 24-hour recalls—were compared. The means were compared and correlation coefficients were determined for protein, milk, eggs, food rich in carotene, and foods rich in ascorbic acid. The seven-day record and the interview of usual food practices gave more similar information as evidenced by correlation coefficients.

For practical purposes, one method should be used in the collection of data for any particular survey. This was done in the present investigation, in which the same interviewer carried out the entire study, and the dietary history interview was used for collecting all data.

Reed and Burke (1954) discussed the reliability of the dietary-intake data for children aged between one to six years, obtained in a longitudinal study of health and development. The research dietary history obtained by personal interview with the child's mother was the instrument employed in this investigation. The reliability of the history was shown to be reproducible within a range of ± 10 grammes of protein per day—that is, a reliability of 71%. It thus appeared from this work that the dietary history made possible the determination of average levels of protein intake " with a reliability found in anthropometric observations ". Good correlations between intake data and rate of muscle growth in the leg indicated that the maternal reporting of the child's diet was accurate for the purposes desired.

The type of dietary history required in such a research project is a detailed account of the normal dietary intake of the individual concerned. There are several methods of collecting this information as set out in the Food and Agriculture Organization's publication " Dietary Surveys ". The method used in this particular survey was (based on the diet history used by Burke (1947). In this method a dietary history is taken by the dietitian, the same interviewer being used throughout the survey, as any variation in interpretation by different individuals is a possible source of error. In the course of the interview two sheets were used to record the information. On the first sheet a detailed account of the food intake over a period of a week was recorded. This included the main meal pattern for the day, including any food usually taken between the main meals. The sheet was divided to include any variations of food intake during pregnancy. The pre-pregnancy diet was used as a basis for the calculations, as it was thought that maternal nutrition did not mean nutrition during pregnancy alone, as " the mother is the product of a lifetime (or more) of nutritional experience " (Thompson, 1956).

The second sheet was used as a means to check information obtained in the first part of the interview. This sheet contained a detailed list of basic foods, and quantities of these foods included daily or weekly were recorded. A third sheet was used for calculation of daily dietary intake.

Three-quarters of an hour to one hour was spent, depending on the patient's ability to give the information required. Before the interview was proceeded with, a detailed explanation of why the patient was being questioned was always given. This made for better relations between the subject and the interviewer, and more reliable information could be gained as a result. The utmost care was taken not to suggest an answer, as this is a possible source of error. At the commencement of the survey a table of normal serves of all foods was drawn up, which was used as a basis for assessing size of meals and individual portions. Visual aids are of great assistance in the questioning of patients. Photographs of normal-sized serves of food were used, but household measures were found to be a much easier way for the patient to express quantities of food consumed.

Many social, economic and racial factors were considered and used to check the patient's statements on food intake—for example, the type of work done by the husband, whether it was shift work, whether and for how long the wife worked during pregnancy, the type of housing (flat, room or house), the amount of money available to be spent on food, time-payment obligations, etc. Because of the number of New Australians included in the survey a knowledge of the dietary patterns of Italian and Greek communities was essential. In assessing the dietary intake of these patients, the length of time they had been resident in Australia was considered. Methods of marketing, type of storage for perishable foods and methods of cooking foods were also considered. Any inconsistency in a patient's statement of dietary intake was regarded with suspicion, and histories not considered reliable were discarded (10).

Analysis of Information

The information from the first and second sheets was transferred to the third sheet, and quantities of food eaten daily were calculated in grammes.

This retrospective method of food analysis can never give more than approximate results. Apart from errors due to personal estimates of approximate food intake, there are likely to be inaccuracies in food tables, and in the estimates of loss of vitamins during cooking. In this study, 15% was deducted from thiamine values to correct for cooking loss, and 50% for vitamin C in cooked foods. However, it is thought that by comparing the results with those of the control series, rather than with an arbitrary table of recommended allowances, these errors have been equalized and largely overcome.

Comparison of the means of height and weight (non-pregnant) in all groups showed no sni-nificant differences between (propositi and controls.

RESULTS

Mongolism

Mothers of mongoloid babies (8) showed no evidence of nutritional deficiency—that is, there were no significant differences between their diets and those of the controls (Table IV). In this table the present scale of recommended daily allowances (Osmond and Wilson, 1954) is shown, together with the findings of a recent nutritional study in pregnancy (Bolton and Forster, 1959).

Spina Bifida

Mothers of babies with spina bifida cystica (13) showed only one positive difference in dietary constituents—namely, vitamin C. However, the estimated vitamin C intake of these mothers (49 mg. per day) is so far above the known minimum human requirement of 10 mg. per day that it is difficult to attach any biological significance to this finding. In the case of other vitamins, the minimum requirement is not yet known, and we are unable to make any such check on the present findings.

Gastro-Intestinal System

Mothers of babies with cleft lip (with or without cleft palate) appeared to have consumed diets that were a little poorer than the controls in thiamine (5% level) (Table IV).

Cardio-Vascular System

Congenital heart defects (11) were suspected in the neonatal period, and the diagnoses were confirmed by follow-up examinations for at least 18 months, and/or by autopsy reports. Mothers of babies with congenital heart defects appeared to have consumed diets that were a little poorer than those of the controls in protein, calories, iron and vitamin C (5% level), and poorer in carbohydrate and niacin (1% level).

Talipes

The diagnosis of talipes in each case was confirmed by follow-up examinations and surgical opinions; this eliminated cases of " pseudo-talipes " or " curled feet ", which are often described as talipes, but which spontaneously correct themselves during infancy. Mothers of babies with talipes (20) appeared to have consumed diets which were little lower than those of the controls in riboflavin, niacin and vitamin C (5% level of significance).

The causation of talipes has for many years been a controversial subject, and the literature does little to clarify it. Some authors, such as Parker and Shattock (1884) and Denis Browne (1959), suggest that moulding of the feet by intrauterine compression is the cause, and it is well known to pædiatricians that babies with this deformity can sometimes be " refolded " into a probable prenatal position, which appears to explain the deviation of the feet. However, it is obvious that this is no universal explanation, as cases occur in which the baby cannot be thus " refolded ", and also cases of talipes are found in fœtuses younger than 20 weeks' gestation, up to which age the fœtus floats freely in the amniotic sac and is not subject

to pressures (Bechtol, 1950). Moreover, dissections of subjects by authors such as Stewart (1951) and Flinchum (1953) reveal abnormalities in tendinous insertions (especially of the tendo Achillis) and unevenness of muscle development in the affected limb, which they consider to be primary to the foot anomaly. These findings have been demonstrated in fœtuses younger than 20 weeks.

In other cases, genetic factors seem occasionally to operate, and ethnic differences in incidence are well described by Stewart. The possible finding here of maternal dietary deficiency in cases of talipes would strengthen the view that there is no one explanation for the deformity.

Other Musculo-Skeletal Defects

In this non-homogeneous group of mothers of the affected babies, 16 appeared to have consumed diets similar to those of the controls.

Miscellaneous Group

Mothers of these babies (14) appeared to have consumed diets which were a little poorer than the controls in iron and thiamine.

TABLE IV

Dietary Analysis (99 Cases)

—	Recommended Allowance (Osmond and Wilson)	Belton and Forster (1959)		Mongolism (8)	Spina Bifida (13)	Cleft Lip (17)	Congenital Heart Defects (11)	Talipes (20)	Musculo-Skeletal (16)	Miscellaneous (14)
Protein (grammes) ..	55	68	$\bar{x}_p$	69	63	60	59	60	60	55
			$\bar{x}_c$	77	67	66	72	69	66	65
			t	0·60	0·84	1·33	2·46[1]	1·78	1·37	1·91
Fat (Grammes) ..	—	—	$\bar{x}_p$	111	111	94	92	121	97	99
			$\bar{x}_c$	119	105	107	106	112	96	106
			t	0·27	−0·45	1·43	1·42	−0·32	−0·15	0·75
Carbohydrate (grammes)	—	—	$\bar{x}_p$	338	324	272	237	272	262	241
			$\bar{x}_c$	279	263	298	320	294	269	244
			t	−1·66	−2·47	1·23	3·48[2]	0·98	0·29	0·17
Calories	2150	—	$\bar{x}_p$	2592	2522	2145	1989	2114	2135	2065
			$\bar{x}_c$	2448	2225	2379	2453	2433	2206	2122
			t	−0·37	−1·56	1·76	2·80[1]	1·82	0·46	0·39
Calcium (grammes) ..	0·8	0·7	$\bar{x}_p$	0·97	0·72	0·80	0·88	0·79	0·89	0·78
			$\bar{x}_c$	1·29	1·00	0·96	1·00	1·01	0·94	0·93
			t	0·71	2·11	1·25	0·68	1·88	0·46	0·92
Iron (milligrammes)	—	9·9	$\bar{x}_p$	10·6	10·2	9·2	8·6	9·3	8·9	8·3
			$\bar{x}_c$	9·6	10·1	10·6	10·7	10·3	9·9	0·6
			t	−1·36	−0·20	2·05	2·26[1]	2·03	1·22	2·35[1]
Vitamin A (thousands of International Units)	5000	3891	$\bar{x}_p$	7·3	6·7	7·2	6·8	6·3	7·3	8·2
			$\bar{x}_c$	5·7	7·8	9·9	8·4	9·3	7·4	7·8
			t	−0·86	0·71	1·74	1·00	2·08	0·02	−0·17
Thiamine (milligrammes)	1·1	0·7	$\bar{x}_p$	0·884	0·780	0·712	0·783	0·754	0·708	0·702
			$\bar{x}_c$	0·816	0·804	0·820	0·942	0·892	0·778	0·858
			t	−0·79	0·31	2·42[1]	1·51	1·63	1·56	2·51[2]
Riboflavin (milligrammes)	1·4	—	$\bar{x}_p$	1·82	1·47	1·36	1·61	1·36	1·49	1·36
			$\bar{x}_c$	2·04	1·78	1·62	1·92	1·77	1·63	1·60
			t	0·48	1·20	1·52	1·08	2·50[1]	0·84	1·01
Niacin (milligrammes)	11·0	—	$\bar{x}_p$	11·2	10·7	9·0	9·0	8·9	9·1	8·7
			$\bar{x}_c$	9·2	10·1	10·3	11·3	10·5	9·9	9·8
			t	−1·89	−0·80	1·96	3·36[2]	2·69[1]	1·05	1·75
Vitamin C (milligrammes)	30	82	$\bar{x}_p$	70	49	65	57	63	64	86
			$\bar{x}_c$	67	82	76	86	85	66	87
			t	−0·66	2·99[1]	1·37	3·03[1]	2·24[1]	0·34	0·03
Degrees of freedom ..				7	12	16	10	19	15	13

[1] Significant at the 5% level.

[2] Significant at the 1% level.

Analysis of Variance

As values of dietary constituents between groups varied for both cases and controls, a further statistical testing (analysis of variance) was carried out as another safeguard. The results are set out in Table V. It will be seen that this indicates some deficiency in the diets of the case mothers in carbohydrate, iron, thiamine, vitamin C (5% level) and niacin (1% level). The trend of this analysis therefore is to agree with the analysis by *t* tests (Table IV).

TABLE V
Analysis of Variance

Nutriment	F.
Protein	1·97 n.s.
Fat	0·25 n.s.
Carbohydrate	2·25[1]
Calories	1·68 n.s.
Calcium	0·71 n.s.
Iron	2·50[1]
Vitamin A and carotene ..	1·37 n.s.
Thiamine	2·45[1]
Riboflavin	1·47 n.s.
Niacin	3·86[2]
Vitamin C	2·10[1]

[1] 5% level of significance, 2·06.
[2] 1% level of significance, 2·74.

DISCUSSION

If it is kept in mind that what is statistically significant is not necessarily biologically significant, it can only be said that the foregoing results suggest the existence of dietary weakness in the mothers of malformed babies. Although the differences are not dramatic, the finding of statistical significance in several constituents, especially in the vitamin B group, is consistent with some degree of hypovitaminosis. There is no evidence of avitaminosis comparable with the experimental deficiencies in the dietary work on animals described earlier.

Of special interest are the thiamine values, which, in common with the figure of Bolton and Forster (0·7 mg. per day), are definitely lower than the present recommended daily allowance of 1·1 mg. Similar findings in the diets of children living in the same city (Melbourne) have also been reported by Cahn and Neal (1959), who found that about one-third of the diets were suboptimal in both niacin and thiamine.

In the present study, there is some evidence of niacin deficiency in the congenital heart disease group, whilst lower figures for thiamine were found in the cleft lip and miscellaneous groups.

The absence of positive significant differences in the mongoloid and spina bifida groups is worthy of comment.

Recent work on chromosomal aberrations in mongoloid subjects has suggested a cytoplasmic basis to this disorder (Jacobs *et alii*, 1959), whilst in this community a fairly strong familial tendency (10·6%) in spina bifida has recently been demonstrated (Durham Smith, 1959). Negative dietary findings in both groups might therefore well have been expected.

ACKNOWLEDGEMENTS

Our thanks are due to Dr. John Colebatch, who proposed and encouraged this study; to Professor Lance Townsend and Dr. John Bolton, for encouragement and advice; to Dr. R. T. Leslie, Miss Betty Laby and Mrs. G. E. MacDonald, of the Department of Statistics, University of Melbourne, for the statistical analyses in this report; to Mrs. Audrey Cahn, of the Department of Biochemistry, for advice on the nutritional findings; and finally to Miss Muriel Ryan, who carefully assisted with the manuscript.

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BACTERIOLOGICAL STUDIES IN CHRONIC BRONCHITIS[1]

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SUMMARY

From patients with chronic bronchitis, either in a small series studied for over a year or in a consecutive series of hospital patients, the common potential pathogens isolated from the sputum were *Hæmophilus influenzæ*, *Streptococcus pneumoniæ* and *Escherichia coli*.

Among 17 patients, from whose sputum 282 cultures were made over a period of 16 months, three were found whose sputum grew *E. coli* as the only likely pathogen, and in two others this organism was isolated with notable consistency.

The frequency with which *E. coli* was found in this study is not solely attributable to previous antibiotic therapy, although influenced by it, and it is considered a significant pathogen in some patients with chronic bronchitis.

It is possible that a variety of organisms usually considered non-pathogenic may play a part in infecting a mucous membrane already damaged by other agents, such as smoking and atmospheric pollution, and that the less susceptible organisms may assume greater significance after the administration of antibiotics.

MANY overseas workers have demonstrated that the significant organisms isolated from patients with chronic bronchitis are *Hæmophilus influenzæ* and *Streptococcus pneumoniæ* (Mulder, 1938, 1940, 1956; Mulder *et alii*, 1952; May, 1953*a*, 1953*b*, 1954; Elmes *et alii*, 1953; Stuart-Harris *et alii*, 1953; Edwards *et alii*, 1957; Murdoch *et alii*, 1960; Cherniack *et alii*, 1959). Although their relationship to exacerbations and to purulence of the sputum is well established, more particularly in relation to *H. influenzæ*, and although the favourable results of antibiotic therapy, reported in a number of careful trials, bear testimony to their clinical importance, the exact significance and role of these organisms in chronic bronchitis is not clear (Edwards *et alii*, 1957; Brumfitt and Willoughby, 1958; Elmes *et alii*, 1959; Glynn, 1959). Certainly it is an over-simplification to regard them as the sole cause, if only because both may be isolated from mucoid sputum between exacerbations. Furthermore, the possibility that other bacteria play a minor part should not be neglected. The present paper describes the bacteriological findings in a series of patients with chronic bronchitis studied over a period of 16 months, and compares the results with the routine findings in hospital in-patients with pulmonary disease. The study was not primarily designed to repeat the detailed correlation of clinical and laboratory findings reported by overseas groups, which are in general agreement, but was planned partly to see whether a similar bacteriological pattern existed in this country, and partly to note the consistency with which organisms were isolated from a particular patient.

PATIENTS AND METHODS

Largely on a basis of their ability and willingness to cooperate, 19 out-patients were chosen for an investigation of the natural history of bronchitis, of which the bacteriological studies to be described formed part. The results of concomitant virological studies have been described elsewhere (Jack and Gandevia, 1960).

Two patients proved unable to take part, and the observations recorded relate to 17 subjects (13 males and four females). All the patients had persistent cough and sputum, which, at least for some part of the period of observation, was purulent or mucopurulent. Chest radiographs, with bronchograms in selected cases, were obtained to exclude other diseases or complications of bronchitis. Exercise tolerance over the series ranged from normal to inability to walk 50 yards on the flat at a slow pace. The clinical features and single-breath tests of ventilatory capacity indicated severe obstructive lung disease in eight patients and less marked airways obstruction in the remainder, always with a significant component reversible by a bronchodilator aerosol. The patients were first examined

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late in 1957, and the bacteriological results extend from this period until February, 1959. Although not included in the tabulated data recorded here, observations on most of the surviving patients continued into 1960. Four patients died within a few weeks of one another during the winter of 1958, all with acute exacerbations of bronchopulmonary symptoms (Jack and Gandevia, 1960).

The patients were interviewed at frequent intervals, and, because of attempts at virus isolation, they were strongly encouraged to report any exacerbation at the first hint of its onset. Among the " hints " stressed to patients were fever, sore or dry throat, rhinorrhœa, increase in cough, sputum volume or purulence, and worsening of wheezing or shortness of breath. It is not surprising, therefore, that " exacerbations " were numerous, that some of these failed to mature and that others were not associated with any objective signs either clinically or on macroscopic or bacteriological examination of the sputum. In these circumstances any detailed attempt to correlate exacerbations and findings is impracticable; when any relationship is mentioned in a specific instance, the exacerbation referred to has been unequivocal and associated with increased sputum purulence.

Sputum specimens collected in the first hour after rising were examined macroscopically each week. Cultures were made on admission to the study, and thereafter at irregular intervals determined by alteration in symptoms or sputum appearance and, in a few instances, by unusual bacteriological findings. Cultures were repeated every week or every fortnight in patients with florid exacerbations. Antibiotics (tetracycline, 2 or 3 grammes per day, and penicillin V by mouth, 2·5 grammes per day, were the only ones used) were given only if the clinical indications were pressing; this policy was deliberately relaxed in the last two months of the survey, when courses of antibiotics were given for several reasons. Nine patients were given 11 courses of one or other antibiotic during 1958, while 12 patients took part in a trial of oral penicillin therapy in December, 1958.

In all, 282 cultures were performed; the minimum number from any patient completing the period of study was seven. From two of the 17 patients, one who died and one who was forced to withdraw because of distance from the hospital, only three and four cultures respectively were obtained.

During a six months' period in 1958, the bacteriological findings were reviewed in patients admitted to hospital for whom a definite diagnosis of lobar pneumonia, bronchopneumonia, chronic bronchitis or aspiration pneumonia (post-operative or post-traumatic) was recorded on the bacteriological request slip accompanying the first sputum specimen. This series represents about half the number of cases from which sputum specimens were cultured, as only in these were unequivocal diagnoses recorded.

In the patients with chronic bronchitis under continuous study, cultures were performed after the sputum had been homogenized by shaking it in its container with sterile glass balls; Dr. Mary Ralston has found this method to compare favourably with multiple sampling of a specimen of unhomogenized sputum (Ralston, personal communication); Elmes *et alii*, 1957). Later in this survey, and in all the routine hospital specimens, a selected purulent portion of the sputum was streaked on the plate with a thick wire loop. Specimens from the bronchitis series were initially inoculated on to horse agar, penicillin chocolate agar and desoxycholate plates, and into serum broth. The routine hospital specimens were inoculated on to horse-blood agar and into serum broth. In recording the results, " *E. coli* " includes all lactose-fermenting coliform organisms unless they were identified by more detailed investigation. Included with *H. influenzæ* are a few strains of *H. hæmolyticus*.

Antibiotic sensitivity tests were performed by a Bondi disc technique (Cowling, 1953).

RESULTS

All the sputum specimens were at least mildly mucopurulent, polymorphs being present on microscopic examination. The bacteriological findings in the chronic bronchitis series observed over 16 months are set out in Table I. Because of the special factors determining the frequency of cultures, the total isolations of any one organism do not necessarily reflect the incidence and persistence of that organism in the series as a whole. *H. influenzæ*, identified in 12 cases, was commonly encountered; in general, these patients were the more severely " bronchitic " (in that they consistently produced sputum specimens of moderate volume and purulence), although not necessarily those with the poorest exercise tolerance. *E. coli* was found in a similar number of patients (13), and was notably persistent in Cases I, IV and XI. In Cases IV, XII and XVI, and perhaps Case VII, it was the only likely pathogen isolated in a total of 37 cultures. Previous antibiotic therapy may have been a factor in

TABLE I

Frequency of Isolation of Potential Pathogens from 17 Patients with Chronic Bronchitis over 16 Months

Case Number	Number of Cultures	Number of "Positive" Cultures[1]	*H. influenzæ*	*E. coli*	*Strep. pneumoniæ*	*Staph. pyogenes*	Other Organisms
I	31	26	17	11	9	—	——
II	29	22	3	6	5	—	*Chromobacterium* (14)
III	28	18	18	—	2	—	——
IV	12	10	—	10	—	—	——
V	7	3	2	—	—	2	——
VI	36	20	2	3	4	—	*Proteus* (11)
VII	9	2	—	1	—	1	——
VIII	11	4	2	1	—	—	*Bact. alkaligenes* (1)
IX	10	5	1	4	—	—	*Streptococcus*, Group A (1)
X	21	17	8	6	1	7	*Proteus* (2), *Streptococcus*, Group A (1)
XI	32	24	8	11	8	8	——
XII	7	1	—	1	—	—	——
XIII	22	18	17	2	—	—	Paracolon bacillus (3)
XIV	3	0	—	—	—	—	——
XV	11	7	2	3	1	—	Paracolon bacillus (1)
XVI	9	2	—	3	—	—	——
XVII	4	2	2	—	—	—	——
Totals ..	282	181	82	62	30	18	34

[1] Cultures yielding one or more of the organisms mentioned in the table.

the isolations from Cases I and XVI, and may have been relevant to 10 of the total of 62 isolations of this organism, most of these following oral penicillin therapy given to 12 patients in December, 1958. *Strep. pneumoniæ* was found in seven cases, in two with some consistency. In Cases X and XI *Staph. pyogenes* was frequently found together with other pathogens. One or more unequivocal clinical exacerbations were noted in relation to the appearance of *H. influenzæ*, *Strep. pneumoniæ* and *E. coli*.

Special interest attaches to Cases II, IV and VI, in which *Chromobacterium prodigiosum*, *E. coli* and *Proteus* species respectively were isolated from persistently mucopurulent sputum over several months. None of these patients had antibiotic therapy within three months of entering the study or during the relevant part of it. The *Chromobacterium* disappeared spontaneously without change in sputum character, and the *Proteus* was insensitive to all antibiotics but streptomycin, which it was not feasible to administer. The *E. coli* in Case IV was grown from mucopurulent and purulent specimens and from one mucoid sample. Although sensitive to tetracycline, it was not eliminated by a ten-day course of this drug, beginning with 3 grammes on each of the first four days; the patient moved to another State shortly afterwards, and no other antibiotic was tried. Persistence of *H. influenzæ* sensitive to tetracycline was also noted occasionally after similar doses of tetracycline and after apparent control of a clinical exacerbation.

Cases XV and XVI are also of interest in relation to the possible pathogenicity of *E. coli*.

CASE XV.—On November 10 and December 1, 1958, as on previous occasions, the patient produced small quantities of mucopurulent sputum yielding *Strep. viridans*. On December 12 a ten-day oral course of penicillin V, 500 mg. five times a day, was begun. On December 15 the sputum was similar in character and quantity, but two forms of *E. coli* were the only organisms isolated. About a week later, at the seaside, the patient developed what she termed "asthma", preceded by a slight sore throat and possibly fever. Within a day or two, the sputum became grossly purulent and greatly increased in amount; culture of a specimen of this type (January 5, 1959) showed *E. coli*, *Strep. viridans* and *Neisseria* species. No eosinophils were present in this sample or in previous ones. The patient described the episode as the most severe attack of bronchitis that she had ever had. Spontaneous return to her former situation took place over the next month.

CASE XVI.—This case followed a similar course. Early in 1958, *E. coli* was transiently identified in the patient's sputum, and *H. influenzæ* was found during a mild exacerbation in August. Subsequently the sputum reverted to its usual mucopurulent appearance, and only *Strep. viridans* was grown. On December 1, 1958, from a similar specimen, *Strep. pneumoniæ* was cultured, and on the next day, with others in the series, she began a ten-day oral course of penicillin V, 500 mg. five times a day. A week later, the sputum was noted to be purulent, and a specimen grew *E. coli* insensitive to all antibiotics tested. On December 17 she was not interviewed, but voluntarily sent in a gargle specimen for virus culture, indicating in her view the onset of an exacerbation. Weekly specimens remained purulent, and culture during January, when she herself felt well again, also showed *E. coli*, this time with different antibiotic sensitivities.

The onset of exacerbations after oral penicillin therapy, in two of 12 patients so treated, associated with the appearance of *E. coli* as the only likely pathogen in the sputum, seems likely to be significant. In a third case (Case VI), *E. coli* appeared in addition to a persistent *H. influenzæ*, and this sputum was recorded as slightly more purulent.

The detailed results relating to the 12 patients given penicillin V by mouth are shown in Table II. In three cases the sputum improved in colour; in two of these *H. influenzæ* had disappeared on sputum culture, and in one *Strep. pneumoniæ* had gone, *H. influenzæ* remaining. In five cases *E. coli* was grown immediately after the course, and in Cases XV and XVI, and possibly also in Case VI, its appearance seemed to produce unequivocal exacerbations. Three patients developed a sore mouth, one of whom also had painful swellings of both submaxillary glands. We can make no direct comparison with a similar series treated at the same time with "Achromycin V", but we have treated most of these patients at some time with tetracycline with more uniform improvement and no such unpleasant side-effects.

We attach little significance to the patients' opinions obtained a month later, if only because it is certain that the opinions of the patients in Cases XV and XVI do not reflect their actual course.

TABLE II

Effect of Oral Course of Penicillin V

Case Number	Patient's Opinion[1]	Our Opinion[2]	Bacteriological Changes
I	No change	Better	*H. influenzæ* disappeared and *E. coli* grown
II	Worse	No change	No change (*E. coli*)
III	Possibly better	Slightly better	*Strep. pneumoniæ* eliminated; *H. influenzæ* remained
IV	No change	No change	No change (*E. coli*)
V	Possibly better	Possibly worse	*H. influenzæ* before; no cultures afterwards
VI	No change	No change	No change (*H. influenzæ*, *E. coli*)
VIII	No change	No change	Transient *E. coli*
IX	Better	No change	No change (*E. coli*)
X	Better	No change	*H. influenzæ* before; culture not repeated
XI	Much better	Much better	*H. influenzæ* and *Strep. pneumoniæ* replaced by *E. coli*
XV[3]	No change	Worse	*Strep. pneumoniæ* eliminated and *E. coli* grown
XVI[3]	Possibly better	No change	*Strep. viridans* only before; *E. coli* after

[1] Patient's opinion, based on well-being, on sputum volume and colour and on severity of cough, and recorded in response to written inquiry a month after the termination of the course.

[2] Our opinion is based on our clinical notes and the sputum colour.

[3] In Cases XV and XVI the patients had exacerbations a few days later; details are recorded in the case reports on page 277. The opinions of these patients a month after the event indicate memory lapses or, more likely, a desire to please the doctors.

Certain organisms are usually not regarded as pathogens when isolated in sputum cultures; but recently Noach (1960) has stressed the frequency with which *N. catarrhalis* was isolated in his series of chronic bronchitic patients, and has suggested that it should be regarded as a potential pathogen. As no other figures for this organism are available, we may note that in the present study *Neisseria* species were isolated from every patient on more than one occasion, and were found in 162 of the 282 cultures. The only organism isolated in every case with comparable frequency was *Strep. viridans* (in 174 of 282 cultures), an organism which Barach (1953) regarded as pathogenic in some cases. These organisms were each found in 70% of the 40 cultures of our patients whose sputum never grew *H. influenzæ*, whilst of the cultures taken from patients whose sputum grew *H. influenzæ* commonly (arbitrarily defined as one-fourth or more of attempts), these two organisms were each found in 55% (of 145 cultures). The difference does not reach statistical significance.

The findings in the several groups of hospital patients are set out in Table III. In the group diagnosed as chronic bronchitis, a variety of organisms was isolated, the common ones being *E. coli*, *Strep. pneumoniæ*, *Staph. pyogenes*, *H. influenzæ* and *Proteus* species. This pattern differs from that of patients with broncho-pneumonia or aspiration pneumonia, in whom *Staph. pyogenes* was found in almost half—that is, about three times the expected incidence in terms of the bronchitis series. The distribution of the other major organisms is similar, although *Strep. pneumoniæ* is a little more common in the bronchitis group. The number of patients in the lobar pneumonia group is too small to justify comparison.

An attempt was made to relate these findings to previous antibiotic therapy, but, as might be expected from a retrospective analysis of hospital records, this was not satisfactory. The culture results recorded relate to the first specimen received, which was as a rule taken before the administration of antibiotics by the hospital staff (except in some of the cases of aspiration pneumonia); but in some instances

TABLE III

Potential Pathogens Isolated from Hospital Patients with Specified Pulmonary Diseases

Disease	Number of Cases	*E. coli*	*Strep. pneumoniæ*	*Staph. pyogenes*	*H. influenzæ*	*Proteus* Species	*Streptococcus* Group A	*Klebsiella*	*Ps. pyocyanea*	*Streptococcus* Group D
Chronic bronchitis	58	16	14	13	10	10	3	2	2	1
Bronchopneumonia	23	8	2	15	2	2	2	—	1	—
Aspiration pneumonia ..	37	12	4	24	6	4	1	—	2	2
Lobar pneumonia	14	2	5	5	2	—	1	—	—	—

TABLE IV

Biochemical Characteristics of Coliform Organisms Isolated from Sputum

Specimen	Indole	Methyl Red	Voges-Proskauer	Eijkman	Growth in Citrate	Comment
1	−	−	−	−	+	Irregular A
2	−	−	−	−	+	Irregular A
3	+	−	+	−	+	*Aerobacter aerogenes* II
4	−	−	+	+	+	Irregular VI
5	+	+	−	−	+	Intermediate II
6	−	−	+	+	+	Irregular VI
7[1]	+	−	+	−	+	*Aerobacter aerogenes* II
8	−	−	−	−	+	Irregular A
9	−	−	+/−	+	+	Irregular VI
10	−	−	−	−	+	Irregular A
11[1]	+	+	−	+	−	*Escherichia coli* Type I

[1] Specimen 7 (*Aerobacter aerogenes* II) and specimen 11 (*Escherichia coli* Type I) were toxic to mice in small dose. Other strains were non-toxic.

it is not clear whether antibiotics had previously been given by the patient's private doctor. The only notable features to emerge from this review were, first, that *E. coli* was isolated from four patients with aspiration pneumonia, from five with bronchopneumonia and from two with chronic bronchitis, none of whom had had any antibiotic therapy for at least a month, and secondly, that the frequency of isolation of *E. coli* was not significantly higher in those patients who had had antibiotics within this period. However, the figures are too small and incomplete to allow us to generalize on either point.

Eleven strains of coliform organisms isolated from patients in our hospital series were examined for us by Dr. Rose Mushin, of the Department of Bacteriology, University of Melbourne (Table IV). They present a variety of biochemical characteristics, and it is of interest that only three were *Klebsiella* species (Irregular VI), and none had the classical *K. pneumoniæ* type of colonial morphology; serological typing was not done.

The results of antibiotic sensitivity tests to the strains found in the chronic bronchitis series under continued observation are set out in Table V; they show no remarkable features. Unequivocal changes occurred only twice in the sensitivity to antibiotics, other than erythromycin, of *H. influenzæ* strains repeatedly isolated from any one patient. Once, in Case III, a strain insensitive to oxytetracycline, which the patient had not been given, was isolated, and once, in Case I, a strain insensitive to chloramphenicol and the tetracyclines was noted. However, considerable variation in sensitivity to erythromycin was found amongst *H. influenzæ* strains isolated from the same patient.

DISCUSSION

We were surprised to find *H. influenzæ* in only 12 of 17 patients with clinically unequivocal chronic bronchitis when repeated cultures were made for over a year. Nevertheless, this incidence is comparable with that revealed by other workers in the United Kingdom, though less than that recorded by May (1953*a*) and Mulder (1956) (80% to 90%). There are various possible explanations. Factors inevitably operative in the selection of a small series of cases may mean that the series is not representative of all patients with chronic bronchitis. Problems of sampling and variations in bacteriological technique (May, 1952; May and Oswald, 1957; Brumfitt *et alii*, 1957; Brumfitt and Willoughby, 1958; Lees and McNaught, 1959*a*) make it difficult to make any precise comparison of results obtained in different centres. It may be relevant that it is our strong impression, supported uniformly by colleagues with special experience in this field in both the United Kingdom and Australia, that the volume of sputum and its degree of purulence are both more striking in bronchitic patients in the former country: certainly the average level in the bedside sputum mug is higher. A final factor may be that *H. influenzæ* is not the dominant

TABLE V

Results of Antibiotic Sensitivity Tests in the Chronic Bronchitis Series[1]

Organism	Penicillin	Streptomycin	Chloramphenicol	Tetracycline	Oxytetracycline	Erythromycin
H. influenzæ	4/54	73/75	75/76	75/76	69/72	38/65
E. coli	0/25	42/49	46/50	39/46	38/47	0/28
Staph. pyogenes	1/14	1/14	14/14	1/14	2/6	13/13
Proteus	0/5	5/5	1/5	0/5	0/5	0/5
Paracolon bacillus	0/4	4/4	4/4	4/4	4/4	0/4
Bact. alkaligenes	—	1/1	1/1	1/1	1/1	0/1

[1] The figures indicate the number of strains sensitive to each antibiotic in relation to the number of strains tested.

pathogen in all cases, a point which may be more apparent when less severe Australian cases are studied.

The possibility that various other organisms, notably *Strep. pneumoniæ*, play some part has been discussed by most of the authors quoted. We are impressed in this connexion by the frequency with which *E. coli* was isolated. In three of our patients, all with mucopurulent sputum, it was indeed the sole likely pathogen, whilst in at least two others it was remarkably persistent. In three of these and in some at least of the hospital series of patients, previous antibiotic therapy in the recent past could not be held responsible for its presence. This is supported by the finding that most of the strains isolated were sensitive to tetracycline, which is the most commonly used antibiotic in these cases. May (1953*a*) states that he frequently isolates the organism, but has not considered it significant. Mulder (1956), who found it rarely except after penicillin treatment, states that its pathogenicity in the respiratory tract "seems to be low". We agree with his statement that sputum containing *E. coli* is often not severely purulent, but this of course is also true of some specimens of sputum containing *H. influenzæ*.

Coliform organisms were isolated "frequently" by Murdoch *et alii* (1959) in 20 of 75 patients observed over six months in winter, the incidence being similar in patients receiving oxytetracycline as in those receiving inert capsules. In a series of 98 patients they found "coliforms and proteus" in about 7% of purulent specimens of sputum, in 4% of mucopurulent specimens and in 7% of mucoid specimens. Lees and McNaught (1959*a*) found coliforms to be the "least infrequent" of potential pathogens other than *H. influenzæ* and pneumonococci; they were isolated from bronchial swabs of three of 28 patients with chronic bronchitis. We have occasionally isolated *E. coli* (and, incidentally, *Proteus* species) as the sole pathogen in routine cultures of bronchial washings. Brumfitt and Willoughby (1958) regarded coliforms as "bronchial pathogens" in four of 37 cases of bronchitis; in two of these *E. coli* was found alone. Aspin and Howells (1960) found that "coliform organisms were predominant" in 27 of 64 severe cases, and Leigh (1960) was similarly impressed by their frequency in patients treated with antibiotics. Murdoch and Gould (1960) comment that in their experience the isolation of coliforms was not related to the clinical state of the patients. The presence of coliform organisms in the upper part of the respiratory tract, a possible source of confusion (as with other organisms), has been noted by us (in the saliva of three of 25 normal subjects), and by Lees and McNaught (1959*a*, 1959*b*). The evidence for the pathogenicity of coliform organisms in chronic bronchitis is thus not conclusive, but we believe that our findings suggest that it may play a significant role at least in this country.

Of the other organisms, it can only be stated that *Proteus* species, paracolon bacilli and *Chromobacterium* were on occasion isolated consistently from patients in the bronchitis series. Streptococci, Groups A and D, were occasional isolates, as was *Pseudomonas pyocyanea*. That even the last-mentioned on occasion may be of some significance is indicated by its appearance in the sputum of several patients in one ward (Cowling, unpublished data), a finding which may have serious implications (Williams *et alii*, 1960).[1]

In the hospital series of patients with chronic bronchitis, the results are in general conformity with the group studied in more detail. Comparison of the bronchitic group with the other groups with specific diagnoses merely serves to emphasize the role of *Staph. pygoenes* in bronchopneumonia and aspiration pneumonia. It does not suggest that this organism plays an important part in chronic bronchitis, although its role in association with viral infections is well established. No demonstrable evidence of infection by a variety of viruses known to affect the respiratory tract was noted in the present series during the year of observation (Jack and Gandevia, 1960).

Whilst the clinical emphasis on *H. influenzæ* and perhaps *Strep. pneumoniæ* as pathogens in chronic bronchitis is justified, we consider that there are some grounds for including a variety of organisms among those pathogenic to a mucous membrane already damaged by other agents, such as smoking and atmospheric pollution, and already the site of catarrhal inflammation (Reid, 1954, 1956). It is reasonable to assume that some of these organisms will have greater significance when other susceptible bacteria are eliminated or suppressed by antibiotics, a point stressed by Barach in 1953. For these reasons, they should not be lightly dismissed, and their further study is warranted.

The therapeutic implications of these findings are reviewed elsewhere (Gandevia and Cowling, in the press).

[1] Since this was written we have had one patient whose purulent or mucopurulent sputum has grown *Pseudomonas pyocyanea* as the only potential pathogen in 10 cultures over three months.

ACKNOWLEDGEMENTS

We are indebted to members of the honorary medical staff of the Royal Melbourne Hospital, who referred patients specially for this study, and to the general practitioners who cooperated in their management. We are grateful to Dr. Rose Mushin, of the Department of Bacteriology in the University of Melbourne, for typing some of the *Bact. coli* organisms and for her valued advice. We are also indebted to Dr. Mary Ralston and the staff of the Clinical Pathology Department for their interest and assistance in this work, and to Sister K. King and the secretarial staff of the Department of Medicine for their administrative help. Our final acknowledgment is to the patients themselves, on whose continued cooperation the study depended.

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LIPID SYNTHESIS IN HUMAN LEUCOCYTES IN ACUTE LEUKÆMIA[1]

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SUMMARY

Lipid synthesis in human leucocytes was examined *in vitro* in blood from patients with acute leukæmia and from normal controls, acetate being used as substrate. The incorporation of acetate-1-C^{14} was measured in total lipids, neutral lipids and phospholipids of the leucocytes and plasma. Both morphological types of acute leukæmia revealed trends away from the normal rates of total lipid synthesis, the rates being high in acute myeloid leukæmia and low in acute lymphatic leukæmia. Fractional lipid synthesis was similar to the normal in acute lymphatic leukæmia, but in the cells of acute myeloid leukæmia proportionally more radioactivity was incorporated into the phospholipid fraction. This difference was not reflected in the plasma-phospholipid fraction. The changes in leucocyte lipid synthesis are discussed in relation to the mixed-cell populations involved and to the question of cell age.

LIPID SYNTHESIS in human whole blood, as revealed by the incorporation of acetate-1-C^{14} into lipids of cells and plasma, has been demonstrated *in vitro* (Altman and Swisher, 1954; Marks *et alii*, 1960; James *et alii*, 1959). The distribution of the labelled lipids in each cell fraction has shown leucocytes to be the principal site of this synthesis (Marks *et alii*, 1960; Pastore and Leonetti, 1958; Elsbach, 1959; Buchanan, 1960). Some incorporation of acetate into the platelet lipids does occur (per platelet, about 1% of that per leucocyte), and although no synthesis of lipids is performed by mature erythrocytes, some residual capacity for lipid synthesis persists in the reticulocytes. Lipids synthesized by the leucocytes are transferred to the plasma, and some at least are then reutilized by the leucocytes (Marks *et alii*, 1960; Lovelock *et alii*, 1960; Rowe, 1960).

Several investigations employing histochemical techniques have obtained data concerning the lipid content of leukæmic cells, but these provide no index of the rate of lipid metabolism (Sheehan, 1939; Boyd, 1936). The in-vitro system employed in the previous studies is eminently suitable for studying the rate of lipid metabolism of leucocytes in various disease states.

The present studies were performed on leucocytes of acute leukæmia obtained from cases of myeloid and lymphatic types and from cases incapable of morphological classification. Incorporation of acetate-1-C^{14} into total lipids and into the neutral lipid and phospholipid fractions of leucocytes and plasma was examined and compared with that in normal subjects.

MATERIALS AND METHODS

Subjects

Studies were performed on the blood of 13 adults in good health and of 13 patients with acute leukæmia. Of the latter group, five had acute myeloid leukæmia, six had acute lymphatic leukæmia and two had leukæmic states of uncertain classification. Those subjects in good health used as controls had normal erythrocyte, reticulocyte, leucocyte and platelet counts and hæmoglobin concentrations. The leukæmic patients were classified according to strict classical criteria on the basis of morphological findings in both peripheral blood and bone-marrow examinations, and when possible this was confirmed at autopsy. Of the two cases unclassified, one was a leukæmia of primitive undifferentiated blast-cell type and the other presented a mixed hæmatological picture. The hæmatological data are listed in Table I.

All leukæmic subjects were investigated prior to commencement of therapy, except one patient (W.R.) of uncertain classification, who had received P^{32} three months previously for lymphosarcoma before the onset of terminal leukæmia.

Incubation Procedure

Ten-millilitre samples of venous blood were drawn into siliconized syringes containing 1 ml. of 2·5% E.D.T.A. (ethylene-diamine-tetra-acetic acid disodium

[1] Received on May 4, 1961.

[2] This work was carried out during the tenure of a grant from the Anti-Cancer Council of Victoria.

TABLE I

Hæmatological Data on Leukæmic Subjects

Subject	Total Leucocytes (Millions per Millilitre)	Lymphoblasts (Percentage)	Lymphocytes (Percentage)	Myeloblasts (Percentage)	Promyelocytes to Metamyelocytes (Percentage)	Neutrophils (Percentage)		Eosinophils, Basophils and Monocytes (Percentage)
						Mature	Band	
Acute myeloid leukæmia :								
H.P. ..	2·0	—	40	10	18	12	16	4
K.P. ..	3·0	—	25	10	5	40	20	—
J.M. ..	48	—	3	80	5	2	10	—
L.H. ..	1·5	—	40	11	17	12	20	—
B.S. ..	40	—	3	8	58	17	14	—
Acute lymphatic leukæmia :								
K.S. ..	70	80	12	—	—	5	2	1
F.H. ..	300	70	22	—	—	6	—	2
B.O. ..	20	32	50	—	—	10	3	5
T.Y. ..	2·5	20	62	—	—	12	—	6
A.C. ..	10	70	22	—	1	3	3	1
G.D. ..	6·0	70	25	—	—	5	—	—
Acute leukæmia Unclassified :								
I.R. ..	2·0	?[1]	44	? 12[1]	8	10	24	2
W.R. ..	288	75	5	20	—	—	—	—

[1] Primitive blasts, unclassified.

salt) in 0·9% sodium chloride solution per 10 ml. of whole blood. The resulting mixtures were added to 25 ml. flasks containing 1 ml. of 0·5M glucose solution and 0·25 ml. of a mixture of sodium acetate-1-C^{14} with non-labelled sodium acetate (0·8M sodium acetate containing 0·46 μc. per millilitre). The flasks were gassed with 100% oxygen, sealed and incubated with shaking for 2·5 hours at 37° C.

Separation of Cells

After incubation the cells and plasma were separated by dextran flotation by the use of a modification of the method of Skoog and Beck (1956) as previously described (Marks *et alii*, 1960). The red cells and platelets were discarded, and the leucocyte and plasma fractions retained for extraction of lipids. The contamination of the leucocyte fraction in normal samples and in leucopenic leukæmic samples averaged three red cells and eight platelets to one white cell, while in those leukæmic samples with high leucocyte counts the contamination was considerably less. On the basis of previous results (Marks *et alii*, 1960), this contamination was considered to be sufficiently low not to interfere with the estimation of leukocyte activity.

Lipid Extraction and Separation

The lipids of leucocytes and plasma were extracted as previously described (Marks *et alii*, 1960) by the method of Folch *et alii* (1957) and separated by Eder's (1958) modification of the silicic-acid-column chromatographic method of Hirsch and Ahrens (1958).

Counting of Radioactivity

The radioactivity of the lipids was assayed in a liquid-scintillation spectrometer employing 0·3% 2,5-diphenyl-oxazole and 0·03% 1,4-bis-2-(5-phenyl-oxazolyl)-benzene in toluene as a solvent-phosphor system. Measurement of radioactivity in this system was with an absolute efficiency of 72% and a background of 35 C.P.M. In all samples 10,000 counts were recorded and all counts were more than twice background. A full discussion and assessment of these methods has been published previously (Marks *et alii*, 1960).

RESULTS

Morphological Grouping

Analysis of the leucocyte counts of subjects with acute myeloid leukæmia (Table I) reveals a considerably varied picture in the peripheral blood. All patients had circulating myeloblasts and other primitive cells of the myeloid series; but the incidence of each particular morphological type of immature cell covered a wide range. All patients showed gross myeloblastic proliferation in their bone marrow; in one instance (patient B.S.) there was also a high percentage of promyelocytes. The subjects with acute lymphatic leukæmia, on the other hand, showed marked central and peripheral proliferation of cells of the lymphatic series, with a considerable proportion of blasts. Of the two unclassified cases, one subject (I.R.) presented a peripheral blood picture consistent with acute myeloid leukæmia, but a bone-marrow smear showing primitive blasts of indeterminate type. The other patient (W.R.), suffering from leukosarcoma terminating in acute leukæmia, was included in the study because he presented an unusual mixed picture with circulating lymphoblasts and myeloblasts.

Normal Values

In these experiments, lipid synthesis was examined only in leucocytes and plasma. Although platelets do synthesize some lipid, it has been shown previously (Marks *et alii*, 1960) that the leucocyte is the major contributor to total lipid synthesis in the system employed. Normal values for incorporation of acetate-1-C^{14} into total lipids of leucocytes and plasma of

normal subjects are shown in Table II. Of the mean total incorporation of 33 $\mu c. \times 10^{-3}/10^{10}$ leucocytes, 75% appeared in the plasma fraction.

Synthesis of Total Lipids in Acute Myeloid Leukæmia

There was a wide variation in the amount of radioactivity appearing in the total lipids of leucocytes and plasma in acute myeloid leukæmia (Table III). However, all values were greater than the highest of the normal controls, in one instance (patient K.P.) being more than twenty-fold greater than the normal mean figure for acetate-1-C^{14} incorporation. There was also a wide variation in the proportion of counts appearing in the plasma. It is evident from a comparison of Tables I and III that a greater proportion of radioactivity was present in the plasma lipids of those samples with the smaller leucocyte counts. No correlation was found between the rate of total lipid synthesis and the proportion of blast cells present.

TABLE II

Incorporation of Acetate-1-C^{14} into Total Lipids of Leucocytes and Plasma of Normal Subjects

	Lipid Synthesis: Leucocytes and Plasma ($\mu c. \times 10^{-3}/10^{10}$ Cells)	Proportion in Plasma (Percentage)
Range of values[1]	23–48	63–80
Mean	33	75

[1] Studies on 13 hæmatologically normal subjects.

TABLE III

Incorporation of Acetate-1-C^{14} into Total Lipids of Leucocytes and Plasma of Subjects with Acute Myeloid Leukæmia

Subject	Lipid Synthesis: Leucocytes and Plasma ($\mu c. \times 10^{-3}/10^{10}$ Cells)	Proportion in Plasma (Percentage)
H.P.	121	80
K.P.	860	94
J.M.	59	46
L.H.	248	84
B.S.	57	26

Synthesis of Total Lipids in Acute Lymphatic Leukæmia

In Table IV are listed the findings in acute lymphatic leukæmia for the estimation of total lipid synthesis occurring in the leucocytes and plasma. The values for the two fractions together were found to be lower than the normal controls, or of the same order as the lowest of these. Again, the proportion of newly-synthesized lipid appearing in the plasma was variable, and was greater the fewer the leucocytes in the incubation system. As in myeloid leukæmia, there was no overt relationship between the rate of lipid synthesis and the proportion of blast cells present.

TABLE IV

Incorporation of Acetate-1-C^{14} into Total Lipids of Leucocytes and Plasma of Subjects with Acute Lymphatic Leukæmia

Subject	Lipid Synthesis: Leucocytes and Plasma ($\mu c. \times 10^{-3}/10^{10}$ Cells)	Proportion in Plasma (Percentage)
K.S.	24	31
F.H.	10	35
B.O.	22	72
T.Y.	14	63
A.C.	24	62
G.D.	19	73

Unclassified Cases

In the first unclassified case (that of I.R.), the acetate-1-C^{14} incorporation into the total lipids of leucocytes and plasma was 176 $\mu c \times 10^{-3}/10^{10}$ leucocytes. This was well above the normal controls and was of the same order as some of the values in acute myeloid leukæmia. The second case (that of W.R.) gave a value of 39 $\mu c. \times 10^{-3}/10^{10}$ leucocytes, within the range of the controls.

TABLE V

Incorporation of Acetate-1-C^{14} into Neutral Lipids and Phospholipids

Subjects	Leucocytes		Plasma	
	Neutral Lipids (Percentage)	Phospholipids (Percentage)	Neutral Lipids (Percentage)	Phospholipids (Percentage)
Normal controls (13)[1]:				
Range	64–75	25–36	73–92	8–27
Mean	69	31	83	17
Acute myeloid leukæmia (5):				
Range	40–49	51–60	70–90	9–30
Mean	44	56	84	16
Acute lymphatic leukæmia (6):				
Range	53–75	25–47	75–89	11–25
Mean	62	38	80	20

[1] Figures in parentheses indicate the number of subjects.

Synthesis of Phospholipids

The proportional synthesis of phospholipids and neutral lipids in normal, acute myeloid and acute lymphatic leukæmia groups is shown in Table V. It is evident that in acute lymphatic leukæmia the percentage of radioactivity incorporated into leucocyte phospholipids as com-

pared with neutral lipids was of the same order as in the controls. Less radioactivity appeared in the phospholipid fraction than in the neutral lipid fraction. However, in acute myeloid leukæmia the incorporation of radioactivity into the leucocyte phospholipids was greater than in the controls and higher than that into the neutral lipids. The percentage of newly-formed phospholipids appearing in the plasma, however, was of the same order in both leukæmic groups as in the normal controls.

DISCUSSION

It is evident in the small group of patients studied that pronounced variations in ability to synthesize lipids occur in the cells of patients with acute leukæmia. The rates of synthesis in mixed-cell populations in acute myeloid leukæmia were increased above the range for the control mixed-leucocyte populations, and some very high rates were observed. On the other hand, lipid synthesis in acute lymphatic leukæmic cells was relatively low, and in some instances was well beneath the range for the controls.

Because of the mixed nature of the cell populations involved, the significance of these variations is difficult to assess. The leucocyte populations of the normal controls contained 20% to 30% of lymphocytes and 68% to 80% of granulocytes, 92% or more of the granulocytes in each case being morphologically mature cells. In the patients with acute myeloid leukæmia, the blood samples employed contained 60% or more cells of the myeloid series, these varying in morphology from myeloblast to band-neutrophil, and this was associated in all cases with rates of lipid synthesis above those of the controls, in some cases markedly so.

Studies on the lipid content of normal and leukæmic leucocytes are few, and such studies as have been done have mostly employed histochemical methods. In 1939, Sheehan reported that normal polymorphonuclear leucocyte granules stained heavily with Sudan Black B, myelocytes moderately, myeloblasts lightly and lymphoblasts not at all. Sudan Black B stains neutral lipids, phospholipids and sterols. These findings were interpreted as suggesting an increase in lipid content with increased maturity of cells of the normal myeloid series. The present studies suggest that the less-mature myeloid cells of leukæmic type possess increased ability for net synthesis of lipids.

Chemical estimations by Boyd (1936) showed a higher content per cell of total lipids and phospholipids in chronic myeloid leukæmia than in normal controls, while both values were lower in chronic lymphatic leukæmia. No estimations have been done in acute leukæmia.

The results in acute lymphatic leukæmia are representative largely of the behaviour of cells of the lymphatic series, for all patients had more than 80% of cells of this series, either blast cells or morphologically more mature forms. Other studies (Kidson *et alii*, 1961) have demonstrated a slightly higher rate of lipid synthesis in normal lymphocytes as compared with normal granulocytes, a fact which suggests that the above figures in acute lymphatic leukæmia represent some degree of depression of the rate of lipid synthesis.

The finding of a high value for total lipid synthesis in one patient (I.R.) of the unclassified group possibly may suggest that his leukæmia is myeloid in type, but no claim can be made for lipid synthesis as a diagnostic tool for distinguishing the type of acute leukæmia. The finding of an intermediate value for the second unclassified subject (W.R.) is consistent with the morphologically mixed picture of lymphoblasts with some myeloblasts in the peripheral blood. This provides confirmatory evidence for the differences in lipid synthesis occurring in the two morphological types of acute leukæmia.

From Tables I and III it is apparent that blood samples with low leucocyte counts (from patients J.P., K.P. and L.H.) showed higher rates of incorporation of acetate-1-C^{14} into lipids than did samples with higher leucocyte counts. From Tables I and IV, however, the lowest levels of acetate incorporation were in one sample (patient F.H.) with a very high leucocyte count and one sample (patient T.Y.) which was leucopenic. It would seem, therefore, that no definite correlation existed between the number of leucocytes present and the rate of incorporation of acetate into lipids.

Studies on the life-span of leucocytes have been reviewed by several authors (Dameshek and Gunz, 1958; Hamilton, 1959). Because of the technical difficulties involved and the problem of assessing extravascular as well as intravascular survival time, no final conclusions are yet possible. Some studies have suggested a shortened life-span for the cells of chronic myeloid leukæmia and a prolonged life-span for those of chronic lymphatic leukæmia (Weisberger and Levine, 1954; Rigas, 1958), but no definitive data are available for acute leukæmia. Craddock and co-workers (1960) have suggested that the leukæmic blast cell is incapable of maturation and cannot be considered to have a "finite" life-span. They obtained data indicating prolonged persistence of leukæmic stem cells, and found the rate of deoxyribonucleic

acid (DNA) synthesis to be much slower in leukæmic cell populations than in normal bone marrow or lymphoid tissue. These findings were confirmed by Gavosto *et alii* (1960), who found thymidine incorporation into leukæmic blast cells to be less than that into normal myeloblasts, this observation suggesting a low order of mitotic activity in the cells of acute myeloid leukæmia.

The present findings of increased lipid synthesis in acute myeloid leukæmia and decreased synthesis in acute lymphatic leukæmia might be explained on the basis of cell age, with increased synthesis in younger cells and diminished synthesis in older cells. Such an explanation would assume the cells in acute myeloid leukæmia to be younger and those in acute lymphatic leukæmia to be older than their mature normal counterparts. However, if Craddock's interpretation of the rates of DNA synthesis is correct, supporting the concept of " maturation arrest " and consequent longer life-span of acute leukæmic cells, then the age of the leukæmic cells is an unlikely explanation. Certainly it would not account for the increased synthesis of lipids in acute myeloid leukæmia.

An alternative hypothesis is that the changes observed in lipid synthesis represent altered patterns of metabolism in abnormal cells rather than variations due to cell age.

In those cases of acute myeloid leukæmia in which rates of lipid synthesis were much increased, and in which there were many cells beyond the myeloblast stage apparently contributing to the observed metabolic pattern, it is tempting to conclude that these morphologically more mature forms were also abnormal cells with altered lipid metabolism. Such an interpretation is not, of course, consistent with the thesis of " maturation arrest " at the blast stage in acute leukæmia. Because of the difficulty of isolating sufficient immature myeloid cells from the normal person, it has not been feasible to test the alternative possibility—that populations of normal, more mature and leukæmic, more primitive cells coexist.

It has been noted that a higher proportion of labelled lipids appeared in the plasma in those cases in which the plasma-cell ratio is higher. The significance of this finding is as yet uncertain; but the possibility that this phenomenon is due to an effect of plasma-protein concentration on the rate of lipid transfer from the cells is being investigated.

The finding of an increased percentage incorporation of radioactivity into the phospholipid fraction in leucocytes was a feature of all cases of acute myeloid leukæmia, despite the wide variation in cellular morphology. This was not the case in acute lymphatic leukæmia, in which the proportional incorporation of acetate into the phospholipid fraction corresponded roughly to the normal. On the other hand, the incorporation of acetate-1-C^{14} into the phospholipid fraction of myeloid leukæmia plasma was similar proportionally to that of normal controls. These results suggest that, while the factors governing the transfer to the plasma of phospholipids and their reutilization by the cells are unaltered in acute myeloid leukæmia, the rate of phospholipid production by the myeloid leukæmic leucocytes is proportionally increased. Reasons for this variation in acute myeloid leukæmia are as yet undetermined. Further studies are in progress concerning the nature of these quantitative changes in phospholipid synthesis and possible qualitative alterations which may be associated with them.

ACKNOWLEDGEMENTS

Gratitude is expressed to Dr. A. J. Barnett and Dr. J. McLean, of the Alfred Hospital, and to Dr. R. Motteram, of the Peter MacCallum Clinic, Melbourne, for permission to study patients under their care, and to the Department of Physiology, University of Melbourne, for use of the liquid scintillation spectrometer for the counting of radioactivity.

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THE EFFECT OF ENZYMIC DIGESTION OF FOODS ON THE LIBERATION OF IRON[1]

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SUMMARY

Raw and cooked liver and cooked blood were submitted *in vitro* to various enzymic digestions, and the amounts of ferrous and total iron released by the treatments were estimated colorimetrically. The action of trypsin alone on cooked blood made available only one-third as much iron as did peptic digestion. Tryptic digestion following peptic digestion did not liberate any further iron than had already been released by peptic digestion alone.

All enzymic digestions performed on cooked liver liberated the same amounts of iron, but the quantity released was less than that obtained from the raw materials by the action of acid and pepsin.

In all treatments the bulk of the iron liberated was in the ferrous state.

IRON in food must be released from its conjugates before it can be absorbed from the gastro-intestinal tract. There is also some evidence that it must be in the reduced ferrous state before it can be assimilated. The percentage of easily available ferrous iron, as well as the total iron, is important when the dietary value of an iron-containing food is assessed. For this reason the role of cooking and intestinal enzymic digestion in releasing iron from its conjugates must be considered.

Kaldor (1957) demonstrated that from 12% to 13% of the total iron in hæmoglobin is liberated by acid peptic digestion of heat-denatured blood, whereas only approximately 0·1% could be extracted with saline from uncooked blood. Brading, Kaldor and George (1957) then showed that more iron is absorbed by rats from cooked than from uncooked blood administered into the stomach. Moore and Dubach (1955) showed that Fe^{59} incorporated into various foods is absorbed from the gastro-intestinal tract.

The effect of cooking and in-vitro acid peptic digestion in releasing iron from conjugates in several foods has been further investigated by Sanford (1960), and these studies have now been extended to determine whether the liberated iron is in the ferrous or ferric state. The effect of tryptic digestion on the liberation of iron from blood and calves' liver has also been studied.

[1] Received on July 6, 1961.

[2] This work has been supported by a grant from the National Health and Medical Research Council of Australia.

METHODS AND MATERIALS

The experiments were carried out under conditions designed to minimize iron contamination. Care was taken that all apparatus and reagents were as free of iron as possible, and all glassware was treated with 3% hydrochloric acid to render it iron-free.

Blood Samples

Blood was obtained from human volunteers. Approximately 10 ml. were collected into a tube containing a small amount of heparin as anticoagulant. The blood was used within six hours of collection. The hæmoglobin content of each sample was determined on three replicate aliquots by the method of Walsh *et alii* (1953), and the iron content was calculated by the use of Hüfner's figure of 0·334% for the iron content of hæmoglobin.

Liver

Half a calf's liver was washed in physiological saline and cut into pieces weighing approximately 5 grammes, and each piece was wrapped in "Cellophane" and stored in a deep freeze cabinet for use as required.

Chemicals[1]

A 3·2N solution of hydrochloric acid in distilled water was used as stock solution.

Commercial pepsin powder conforming to the specifications of the British Pharmacopœia (Parke Davis "Pepsin 1 : 2500") was used. As required, aliquots were weighed immediately before use and added in the powder form.

A 9·6N solution of sodium hydroxide in distilled water was used.

A 0·83M solution of sodium bicarbonate was made up freshly immediately before use.

A stock solution of 1% trypsin ("DIFCO" certified 1 : 250) was made up in N/20 hydrochloric acid and stored at 4° C.

[1] The grade used was Analytical Reagents (A.R.) except where stated.

A 0·01% solution of both disodium hydrogen phosphate (Na_2HPO_4) and a 0·01% solution of potassium dihydrogen phosphate (KH_2PO_4) were prepared in distilled water and kept as stock. The phosphate ion solution used was in a ratio of 8·8 ml. of potassium solution to 1·2 ml. of the sodium solution.[1]

Cooking and Digestion of Blood

All experiments were performed in triplicate. One millilitre of blood was pipetted into a 25 ml. volumetric flask containing 5 ml. of saline, and the flask was placed in a waterbath at 95° C. for 30 minutes. The flask was agitated continually during the first five minutes of immersion and thereafter at ten-minute intervals. This constituted the cooking procedure.

The cooked blood was treated (*a*) by acid peptic digestion alone, (*b*) by tryptic digestion alone, and (*c*) by acid peptic digestion with subsequent tryptic digestion.

For the peptic digestion, hydrochloric acid and pepsin were added to give a final concentration of 0·32N hydrochloric acid and 0·64% pepsin; saline was added to a total volume of 25 ml. The pH was found to be 1·5. In the second series of experiments, trypsin, sodium bicarbonate solution and phosphate ion solution were added to a final concentration of 0·1%, 0·1M and $1·2 \times 10^{-4}$M respectively. The pH was adjusted to between 7·5 and 7·8 with the sodium hydroxide solution and the volume was made up to 25 ml. For the third experiment (peptic followed by tryptic digestion) the volume of blood, hydrochloric acid and pepsin was made up to 15 ml. with saline, and after one hour at 37° C., trypsin, sodium bicarbonate and phosphate ion solutions were added. After adjustment of the pH to between 6·0 and 6·5, the final volume was made up to 25 ml. All digestions were performed for one hour at 37° C.

Digestion of Liver

A known weight (approximately 2 grammes) of liver was macerated by mortar and pestle, transferred quantitatively to a 100 ml. flask, and made up to volume with saline. The total iron content was determined on four ten-millimetre aliquots of each suspension. The aliquots were "wet-ashed" in a Kjeldahl flask with concentrated sulphuric and perchloric acids, and the amount of iron present was measured by the orthophenanthroline absorptiometric method.

The non-hæmatin iron—that is, the ferritin and hæmosiderin iron—was measured on triplicate ten-millilitre aliquots of the suspension of liver tissue by the method described by Kaldor (1954). This method precipitates the bulk of the hæmoglobin by adding 10% hydrochloric acid followed by 40% trichloroacetic acid. The ferritin and hæmosiderin iron are liberated by the procedure with only a small amount of the hæmoglobin iron. The iron in the supernatant solution is measured with orthophenanthroline and a correction is made for the hæmoglobin iron liberated by the acidification. For the saline extracts, 6 ml. aliquots of the liver suspension were transferred to 25 ml. volumetric flasks, cooked as required and made up to 25 ml. with physiological saline. The flasks were incubated as for enzymic digestion of blood.

The enzymic digestions of cooked and uncooked liver were performed on 6 ml. aliquots of the liver suspensions in the same manner as the digestions of blood.

Extraction and Assay of the Liberated Iron

On completion of each digestion, the contents of the flask were transferred to a tube and centrifuged at 2000 *g* for 30 minutes. An aliquot of 20 ml. of the supernatant solution was pipetted into a tube, 4 ml. of 40% trichloroacetic acid solution were added, and the tube was placed in a waterbath at 90° C. for 15 minutes to precipitate the protein. The tube was then centrifuged at 600 *g* for 30 minutes.

Iron assay was performed on triplicate aliquots of the supernatant solution obtained. An absorptiometric method described by Kaldor (1953) using 1 : 10 orthophenanthroline reagent for determination of iron extracted from serum was adapted for the present purpose. The pH was adjusted to 4·6, a reducing agent was added to convert any ferric iron to the ferrous state, and orthophenanthroline was added to produce a coloured complex with the iron liberated. Triplicate estimations were also performed on further aliquots of each supernate, but the reducing agent was not added. Only the ferrous iron liberated by the digestion was measured in these estimations. The difference between these results and the total iron in the supernates was taken to be the amount of ferric iron present. Blank solutions were used to correct for colour in the supernatant fluid and for colour and iron in the reagents used.

[1] Concentrations of sodium bicarbonate, trypsin and phosphate ion solutions used in the experimental procedures were calculated from figures in the "Handbook of Biological Data" (1956) for trypsin and sodium, potassium, bicarbonate and phosphate ions found in normal human duodenal and pancreatic secretions. The pH at which various digestions were carried out were the pH values of the stomach, duodenum and pancreatic secretions given in this handbook.

TABLE I

Liberation of Iron from Cooked Blood

Treatment	Oxidation State of Iron Estimated	Number of Assays	Mean Total Iron Content (μg. Fe per ml. Blood)	Mean Liberated Iron (μg. Fe per ml. Blood)	Percentage Total Iron Liberated (±S.D.)
Acid-peptic digestion	Fe^{++}	11	530	50·8	9·5±0·62
	$Fe^{++}+Fe^{+++}$	10	530	62·8	11·7±0·30
Acid-peptic digestion plus tryptic digestion	Fe^{++}	15	505	48·1	9·4±0·24
	$Fe^{++}+Fe^{+++}$	15	505	63·3	12·4±0·84
Tryptic digestion alone	Fe^{++}	12	485	18·4	3·8±0·38
	$Fe^{++}+Fe^{+++}$	12	485	23·9	4·9±0·56

TABLE II

Liberation of Iron from Liver

Treatment	Number of Assays	Mean Total Iron Content (μg. Fe per Gramme of Liver)	Mean Liberated Iron (μg. Fe per Gramme of Liver)	Percentage Total Iron Liberated	Mean Tissue Iron Content (μg. Fe per Gramme of Liver)	Mean Tissue Iron Liberated (μg. Fe per Gramme of Liver)	Percentage Tissue Iron Liberated (±S.D.)
Saline extraction of raw liver ..	3	87·8	17·6	20·1	33·9	17·0	50·1± 4·7
Acid-peptic digestion of raw liver	4	87·8	30·1	34·3	33·9	28·8	84·9± 6·1
Saline extraction of cooked liver	1	87·8	7·4	8·4	33·9	6·6	19·4
Acid-peptic digestion of cooked liver	4	87·8	18·3	20·8	33·9	12·0	35·2±15·7
Tryptic digestion of cooked liver	5	87·8	15·6	17·8	33·9	13·0	38·2±13·6
Acid-peptic digestion of cooked liver	16	113·1	24·9	22·0	30·8	15·3	49·7±31·9
Acid-peptic digestion plus tryptic digestion of cooked liver ..	12	113·1	21·9	19·3	30·8	11·7	37·9±25·4

RESULTS

Blood

The results are shown in Table I. The mean percentage of iron liberated from denatured hæmoglobin by acid peptic digestion was 11·7%. This is approximately the same as was found by Kaldor (1957). Most of the liberated iron (81%) was in the ferrous state. Tryptic digestion of denatured hæmoglobin following peptic digestion did not significantly increase the amount of iron released from its conjugates. The ratio of ferrous to ferric iron was only slightly less than with peptic digestion alone. Tryptic digestion alone released only 4·9% of the total iron, with a similar ratio of the two forms. It is to be noted that in earlier work (Sanford, 1960) saline extraction of cooked hæmoglobin yielded 1·8% of the iron. This means that the tryptic digestion in the present experiment probably released only about 3% of the iron or about one-third of that released by the peptic digestion alone.

Liver

The results are shown in Table II. The amount of iron released from the tissue iron component was calculated as follows:

Total iron—Tissue iron = Hæmoglobin iron
(Total Fe) (Tissue Fe) (Hb. Fe)

$$\text{Iron released from Hb. Fe} = \text{Hb Fe} \times \frac{X}{100}$$

where X is the percentage of iron released from hæmoglobin under comparable conditions, as found by Sandford (1960) and in Table I.

Iron released from Tissue Fe = Total Fe released—Fe released from Hb Fe.

Saline extraction of raw liver yielded 20·1% of the total iron and a calculated 50·1% of the tissue iron. Acid peptic digestion of the raw liver increased these percentages to 34·3 and 84·9 respectively.

Only one estimation was made of the iron extracted with saline from cooked liver, and a value of 8·4% of the total iron was obtained. This was comparable with the results of previous experiments (Sanford, 1960) and was considerably less than the 20·1% extracted from raw liver. The amounts released from the total and the tissue iron of cooked liver by acid peptic digestion were also significantly less than the amounts released from raw liver by acid peptic digestion. Tryptic digestion of cooked liver released approximately the same amount of iron as peptic digestion.

The possibility that tryptic digestion following peptic digestion might release more iron was investigated on a different liver. The results in the last two rows of Table II show that peptic digestion released 22·0% and peptic followed by tryptic digestion 19·3% of the total iron. Figures for released iron quoted in Table II represent the total iron released. Estimations of the ferrous iron alone and of the total iron were performed, but no significant differences were found, so that all the iron released was in the ferrous state.

DISCUSSION

The results indicate that *in vitro* the peptic digestion of cooked blood is the important factor in liberating iron. Tryptic digestion has some effect on cooked blood which has not been previously digested with pepsin, but does not increase the availability of iron from cooked blood which has already been digested with pepsin. The relative ineffectiveness of trypsin probably explains the observation of Pirzio-Biroli *et alii* (1958) that, after gastrectomy,

patients are able to absorb iron salts but do not absorb food iron as well as do normal subjects. Further work to be reported separately has shown that the iron liberated by peptic digestion of cooked blood or cooked liver is absorbed *in vivo* by rats, and that there is little absorption of iron from the residues of the digests of these substances. The high percentage of ferrous iron liberated by peptic digestion probably facilitates absorption (Venkatachalam *et alii*, 1956).

The lower pH at which the action of trypsin was examined (pH 6 to 6·5 for trypsin following acid and pepsin, 7·5 to 7·8 for trypsin alone) apparently had no effect either on the iron already released by pepsin or on the liberation of iron by trypsin alone. This was unexpected in view of the statement by Groen *et alii* (1947) that iron is not soluble at pH values above six. However, the concentration of the ferrous iron present at the higher pH values did not exceed the solubility products of the hydroxide or phosphate. The small amount of ferric iron may have been present in a conjugated state, but this was not investigated.

Saline extraction of raw liver was assumed by Kaldor (1958) to remove the ferritin component of the tissue iron, but to leave the hæmosiderin component. The extraction of a calculated 50% of the tissue iron is somewhat less than was found by Kaldor, but this may be due to a species difference or to the ferritin content of the particular calf's liver used. Furthermore, Kaldor's treatment of the saline extract was designed to measure the total iron in the extract, whereas that used in the present work was intended to measure as far as possible only the liberated ionic iron. Kaldor used distilled water instead of saline for his extraction and heated the extract with 10% hydrochloric acid for 30 minutes at 90° C. Peptic digestion of the liver suspension significantly increased the iron liberated.

On the other hand, preliminary cooking reduced the amount of iron which could be extracted with saline or by peptic digestion. This is the reverse of the situation found with blood (Kaldor, 1957; Sanford, 1960), in which cooking facilitated the liberation of iron both by saline and by peptic digestion. The mechanism of the action of heat in liver tissue has not been investigated, and it is not known whether denaturation of the liver-cell structures or of ferritin itself prevents the release of iron. It is of interest, however, that in the early days of pernicious anæmia treatment with liver it was thought essential that the liver be raw. Cooked liver did not give the same response as raw liver. It is now known that the active principle (vitamin B_{12}) is heat stable in aqueous solution, and it is likely that cooking merely prevented release of the vitamin from the tissues as it does the release of iron.

All the iron in the extracts and digests of liver tissue was in the ferrous form. As ferritin iron is in the ferric state (Granick, 1947), reduction must have occurred even in the saline extracts of raw liver. The mechanism of this reduction has not been investigated, but it has been found that the commercial pepsin preparation used contained sufficient reducing agent to reduce all the iron liberated from both blood and liver. However, this is not the explanation of the ferrous iron found in the saline extracts of liver tissue, which must have been reduced by agents in the tissue itself.

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FELTY'S SYNDROME[1]

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SUMMARY

The clinical and hæmatological features of eight cases of Felty's syndrome are described, and are compared with those of other cases reported in the English literature.

The three principal features are chronic rheumatoid arthritis, splenomegaly and neutropenia; other less common features include hepatomegaly, pigmentation, lymph-node enlargement and chronic leg ulceration. Infections are common, and are often recurrent and prolonged.

The neutropenia is marked, counts of less than 1000 per cubic millimetre being the rule; it is usually unaffected by infection. A mild anæmia is common, and occasionally an autoimmune acquired hæmolytic anæmia is associated. Symptomless thrombocytopenia may occur. The marrow is usually hyperplastic, but "maturation arrest" of the granulocytic series is not common.

The administration of adrenocortical steroids in general causes either no increase in white cells, or a temporary increase which is not sustained despite continued treatment. It is concluded that the administration of steroids is not a satisfactory treatment for the neutropenia of Felty's syndrome.

Splenectomy usually results in permanent increase in neutrophils. The importance of distinguishing between short-term and long-term response is stressed. In the great majority of cases there is an immediate increase in neutrophils to normal or above normal counts, the maximum count occurring within two to seven days. Subsequently the count falls; usually it remains within normal limits, but in some cases it falls to presplenectomy or lower-than-normal levels after weeks or months. Thus splenectomy cannot be said to be successful until the patient has been followed for at least many months.

IN 1924 Felty described five cases of an unusual clinical syndrome, "chronic arthritis in the adult, associated with splenomegaly and leucopenia". In addition to the three principal features—namely, chronic rheumatoid arthritis, splenomegaly and neutropenia—all his patients gave a history of loss of weight, all were undernourished, and all showed a yellowish-brown pigmentation of the skin most prominent on the exposed surfaces; some showed slight enlargement of superficial lymph nodes, and all but one had mild anæmia. Another clinical feature described more recently has been leg ulceration (Smith and McCabe, 1948; Peden, 1949). In addition, it has been recognized that in a few cases of Felty's syndrome an autoimmune hæmolytic anæmia is associated (de Gruchy, 1954) and that thrombocytopenia, usually symptomless, is sometimes present.

Since Felty's original description, only a relatively small number of cases has been described in the English literature. The purpose of this paper is to record briefly eight patients with Felty's syndrome studied by the authors, and to discuss the clinical and hæmatological features of the disorder with particular reference to the response to adrenocortical steroid administration and to splenectomy.

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CLINICAL FEATURES

THE clinical features of the eight patients are summarized in Table I.

AGE AND SEX DISTRIBUTION

The ages of our patients at the time of diagnosis ranged from 43 to 75 years, seven being aged over 60 years. There were six females and two males.

ARTHRITIS

The duration of arthritis before diagnosis of Felty's syndrome ranged from five to 30 years, with a mean of 16 years. At the time of diagnosis there was active polyarthritis in four cases; in the other four cases there was only residual deformity without polyarthritis. The polyarthritis was moderately severe in two cases and was mild in two. The involved joints are listed in Table I.

SPLENOMEGALY

The spleen was clinically palpable in seven of eight cases; in Case II it was not palpable on repeated examination, but at operation it was three times normal size.

INFECTIONS

Infections occurred in six cases. The infections were commonly recurrent, frequently prolonged and sometimes at multiple sites. Infection was the immediate cause of death in

TABLE I

Clinical Features

Patient's Case Number, Age (Years) and Sex	Arthritis				Infections	Spleen[2]	Other Features
	Duration before Diagnosis (Years)	Joints Involved[1]	Deformity	Clinical Course			
I : 43 : M.	6	P.I.P. M.T.P. M.C.P. Wrists Elbows Knees	Marked deformity of hands. Synovial thickening of wrists Ankylosis of elbows Synovial thickening of knees, marked deformity of feet	Two episodes of severe polyarthritis 4 and 6 years prior to presentation. Moderate polyarthritis time of presentation	Two episodes of pneumonia. One episode of pericarditis	10 cm. below L.C.M.	Many subcutaneous nodules. X-ray examination of hands: osteoporosis, ulnar deviation, subluxation of M.C.P. joints, periarticular erosions. Latex fixation strongly positive
II : 66 : M.	6	P.I.P. M.C.P. M.T.P.	Mild fusiform swelling interphalangeal joints, and mild deformity of feet	Initially mild polyarthritis. Residual deformity only for several years prior to presentation	Recurrent episodes of bronchitis for several years. One attack of tonsillitis. Ischiorectal abscess with slow response to treatment (Figure III)	Repeatedly impalpable	—
III : 65 : F.	20	P.I.P. M.C.P. M.T.P.	Gross deformity of hands and feet	Mild polyarthritis for 10 years following onset. Residual deformity only for 10 years prior to presentation	One episode of maxillary sinusitis lasting five weeks prior to admission. Large furuncle with cellulitis on leg following mosquito bite. Slow response to treatment	2 cm. below L.C.M.	X-ray examination of hands: osteoporosis, ulnar deviation, subluxation of M.C.P. joints, periarticular erosions. Result of latex fixation test strongly positive
IV : 68 : F.	18	P.I.P. M.C.P. Wrists	Moderate deformity of hands, slight swelling of the wrists with tenderness	Recurrent remissions and exacerbations for 18 years. Mild polyarthritis at time of presentation	Sore throat lasting two weeks. Recurrent crops of persistent boils. Several attacks of bronchitis. One episode of pneumonia	6 cm. below L.C.M.	—
V : 63 : F.	28	P.I.P. M.T.P. Hips Right elbow	Moderate deformity of hands. Slight deformity of feet	Mild intermittent polyarthritis for 8 years. Mild polyarthritis at time of presentation	None	6 cm. below L.C.M.	X-ray examination of hands: osteoporosis, ulnar deviation, subluxation of M.C.P. joints
VI : 75 : F.	30	P.I.P. M.C.P. Wrists Knees	Moderate deformity of hands and feet	Previous history unreliable. No recent polyarthritis	Diverticulitis with abscess and vesico-colic fistula. Developed pneumonia, pyæmic abscess and died	7 cm. below L.C.M.	Typical joint changes *post mortem*
VII : 70 : F.	17	P.I.P. M.C.P. Wrists Feet	Marked deformity of hands and feet. Mild thickening of synovial membrane of wrist	Intermittent mild polyarthritis for 5 years. Residual deformity only for 12 years	None	6 cm. below L.C.M.	Chronic leg ulcer 12 years. Sjögren's syndrome. X-ray examination of hands: osteoporosis, ulnar deviation, subluxation of M.C.P. joints. Result of latex fixation test negative
VIII : 72 : F.	5	P.I.P. M.C.P. Wrists Knees	Moderate deformity of hands	Moderate polyarthritis for 5 years	Bilateral bronchopneumonia	2 cm. below L.C.M.	—

[1] P.I.P., proximal interphalangeal joints; M.C.P., metacarpo-phalangeal joints; M.T.P., metatarso-phalangeal joints.
[2] L.C.M., left costal margin.

TABLE II

Hæmatological Findings at Diagnosis

Case Number	Hæmoglobin Value (Grammes per 100 ml.)	White Cell Count (per c.mm.)	Neutrophil Count (per c.mm.)	Platelet Count (per c.mm.)	Reticulocytes (Percentage)	Erythrocyte Sedimentation Rate, Westergren (mm. per hour)	Result of Antiglobulin (Coombs) Test	Result of L.E. Cell Test
I	5·2	1200	672	140,000	6·1	125	Positive	Negative
II	13·5	1920	280	197,000	1·3	10	Negative	Negative
III	12·1	2500	275	270,000	2·7	104	Negative	Negative
IV	12·7	2400	816	76,000	4·2	27	Positive	Negative
V	12·7	2000	1020	140,000	2·9	26	Negative	Negative
VI	7·7	950	551	151,000	0·5	28	Negative	Negative
VII	10·8	1700	425	189,000	1·8	100	Negative	Negative
VIII	12·1	1500	300	346,000	—	—	—	—

one case (Case VI). Infections in the other cases were often severe, but responded to appropriate antibiotic therapy and to surgery when indicated. In two cases no infections occurred despite severe neutropenia.

OTHER FEATURES

Generalized pigmentation of the skin and significant lymph-node enlargement were not present in any case. Hepatomegaly was present in four cases. In one case (Case VII) there was a large chronic ulcer, measuring 17·5 by 10 cm., in the region of the medial malleolus of the right leg; there was extensive pigmentation of the lower half of the left leg. The ulcer had been present for 12 years.

HÆMATOLOGICAL FINDINGS

PERIPHERAL BLOOD

The peripheral blood findings are summarized in Table II.

Leucopenia

The total leucocyte counts at the time of diagnosis ranged from 950 to 2500 per cubic millimetre; the neutrophil counts ranged from 275 to 1020 per cubic millimetre. However, during the time when patients were under observation, the counts in some cases fell lower—for example, in Case I (Figure I). A differential leucocyte count showed a mild "shift to the left" in four cases. In some cases there was a reduction in the absolute number of monocytes and lymphocytes. In no case was there any increase of lymphocytes or monocytes, but in some instances the absolute number was increased after splenectomy (see section under "Splenectomy"). In no case was there an increase in the absolute eosinophil count. In Case V white-cell counts were available for 16 years prior to diagnosis (Figure VI); it will be noted that, with the exception of two counts, they were all within the normal range, usually at the lower limit; definite neutropenia developed after 16 years' observation.

Neutrophil Response to Infection

In six cases neutrophil counts were performed during an infection; in none did the infection result in any significant increase in the count.

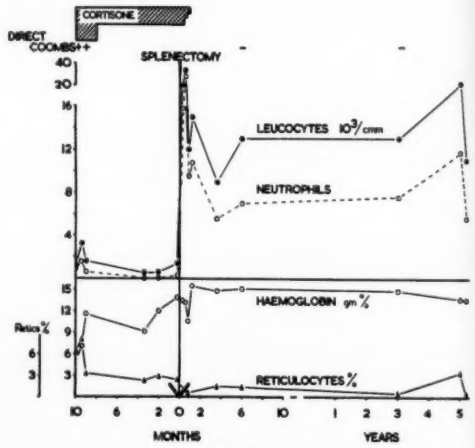

FIGURE I

Case I, showing absence of neutrophil response to cortisone, and sustained response following splenectomy. The initial dose of cortisone was 150 mg.

The lack of neutrophil response was most strikingly shown in Cases II and VI. The first patient (Case II) presented with remittent fever (temperature up to 103° F.) and was found to have extensive cellulitis of the ischio-rectal area which subsequently went on to abscess formation; despite this severe infection, the

total neutrophil count did not rise above 200 per cubic millimetre (Figure II). The other patient (Case VI) died of pyæmia, but her neutrophil count did not exceed 880 per cubic millimetre.

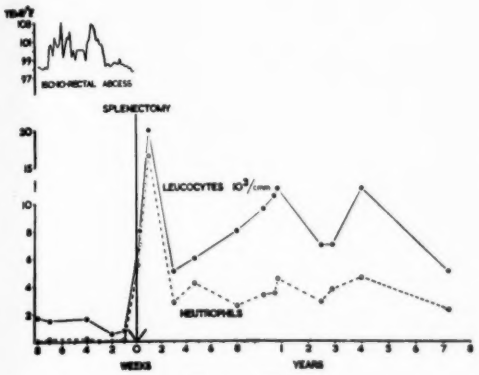

FIGURE II

Case II, showing absence of neutrophil response to infection, and sustained response following splenectomy

Anæmia

Three patients were anæmic at the time of diagnosis; three others became anæmic under observation. Two patients (Cases I and IV) had overt auto-immune acquired hæmolytic anæmia with a positive response to the anti-globulin (Coombs) test.

Thrombocytopenia

In five cases the platelet count was within normal limits (lower normal in our laboratory is 150,000 per cubic millimetre), and in three it was moderately reduced. However, in no case was there clinical bleeding.

Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate of seven patients was estimated; it was slightly raised in three, markedly raised in three, and normal in one.

BONE MARROW

The bone-marrow findings in seven cases are summarized in Table III. In five the marrow was hypercellular and in two normocellular. Granulopoiesis was hyperplastic in all five hypercellular specimens. In only one of the seven cases was there a "shift to the left" with a relative paucity of marrow cells beyond the myelocyte stage (that is, of metamyelocytes and stab forms) giving the picture of so-called "maturation arrest"; this patient (Case III) had normocellular marrow. Erythropoiesis was hyperplastic in five of seven specimens examined; this included the two patients with auto-immune acquired hæmolytic anæmia. Megakaryocytes were present in at least normal numbers in six cases and were definitely increased in one (Case II); in all cases they were morphologically normal.

RESULTS OF TREATMENT

ADRENOCORTICAL STEROID HORMONE ADMINISTRATION

Adrenocortical steroid hormones were administered to three patients.

In Case I, the patient (who also had an auto-immune acquired hæmolytic anæmia) was given 150 mg. of cortisone daily for eight weeks, and then 50 mg. daily for a further eight months. There was a temporary rise in both the total leucocyte count and the neutrophil count, which rose to 3200 and 1728 per cubic millimetre respectively in eight days, then falling to pre-treatment levels of 1600 and 704 per cubic millimetre respectively, despite the continued administration of cortisone; although the neutropenia was not relieved, there was a

TABLE III

Bone-Marrow Findings

Case Number	Cellularity	Granulopoiesis	Erythropoiesis	Thrombopoiesis
I	Markedly hypercellular	Moderately hyperplastic Normal maturation	Markedly hyperplastic Macro-normoblastic	Normal
II	Moderately hypercellular	Slightly hyperplastic Normal maturation	Slightly hyperplastic Normoblastic	Moderate increase in megakaryocytes
III	Normocellular	Normocellular Maturation arrest	Normocellular Normoblastic	Normal
IV	Moderately hypercellular	Slightly hyperplastic Normal maturation	Moderately hyperplastic Normoblastic	Normal
VI	Markedly hypercellular	Moderately hyperplastic Normal maturation	Moderately hyperplastic Normoblastic	Normal
VII	Moderately hypercellular	Moderately hyperplastic Normal maturation	Slightly hyperplastic Normoblastic	Normal
VIII	Normocellular	Normocellular Normal maturation	Normocellular Normoblastic	Normal

significant amelioration of the hæmolytic anæmia (Figure I).

The second patient (Case II) was given a twelve-day course of 120 units of ACTH per day, without any change in either total leucocyte or neutrophil count. A marrow examination was performed after seven days' treatment with ACTH; there was no significant change except a slight increase in erythropoiesis.

The third patient (Case IV), who also had an auto-immune acquired hæmolytic anæmia, was treated with long-acting ACTH in a dosage of 10 units daily for 10 days, and then with cortisone, 100 mg. daily for 14 days. There was no response to ACTH but a rise in both the total leucocyte count and the neutrophil count to 7900 and 2880 per cm. respectively, after 12 days of cortisone treatment. Splenectomy was then performed because of the hæmolytic process, so that it was not possible to observe the leucocyte response following cessation of cortisone therapy.

SPLENECTOMY

Splenectomy was performed on six patients. In two (Cases II and III) the indication was recurrent infection, while in a further two (Cases I and IV) the indication was recurrent infection and associated acquired hæmolytic anæmia. A fifth patient (Case V) suffered from marked lassitude, in the absence of anæmia, and out of proportion to the severity of her polyarthritis; it was thought that neutropenia *per se* was contributing to the lassitude. In Case VII splenectomy was performed in the hope that ulceration of the leg, which had been present for 12 years, would heal to the point where skin grafting would be possible.

The effect of splenectomy has been considered in relation to both the short-term and the long-term response (Table IV).

Short-Term Response

In five cases white-cell counts were performed within hours or days of splenectomy. In all cases there was a prompt rise in both total leucocyte and neutrophil counts, maximum counts being reached in from 36 hours to seven days; in four of the five cases counts rose to above normal values.

In Cases I and VII counts were performed within minutes or hours of splenectomy. Figure III shows the immediate response in Case I. Within 15 minutes a definite increase in total count had occurred, owing mainly to an increase in lymphocytes; at 90 minutes the neutrophil count had risen to 1900 per cubic millimetre (pre-operative figure, 365 per cubic millimetre), and at 24 hours it had reached 16,000 per cubic millimetre. Figure VII shows the response in Case VII; before operation the total leucocyte count

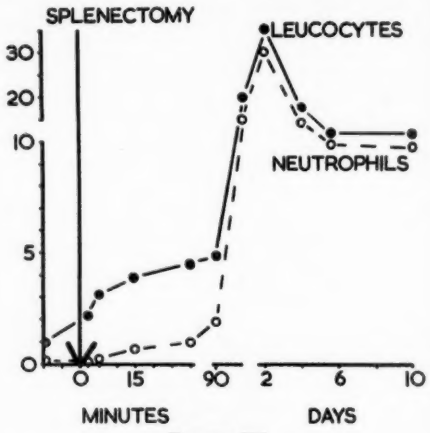

FIGURE III

Case I, showing immediate response to splenectomy. Within 15 minutes a definite increase in total count had occurred, due mainly to lymphocytes. At 90 minutes the neutrophil count had risen to 1900 per cubic millimetre and at 24 hours to 16,000 per cubic millimetre

was 1700 per cubic millimetre and the neutrophil count 425 per cubic millimetre. Two counts were performed on the operating table, one after anæsthesia had been induced and a second after the abdomen was opened, but before ligation of the splenic pedicle; both these counts showed a significant rise, the leucocyte and neutrophil counts being 5000 and 1500 per cubic millimetre respectively. A further count was performed just after the pedicle had been clamped; there was no significant increase. However, at 30 minutes after splenectomy the counts had risen to the normal range, and the maximum count was reached at 36 hours.

TABLE IV

Effect of Splenectomy on White-Cell and Neutrophil Counts

Case Number	Short-Term Response			Long-Term Response		
	Time of Maximum Count	Maximum White-Cell Count (per c.mm.)	Maximum Neutrophil Count (per c.mm.)	Time of Follow-up	White-Cell Count (per c.mm.)	Neutrophil Count (per c.mm.)
I	2 days	35,000	29,400	5 years (alive)	11,000	5600
II	7 days	19,990	16,150	7 years (dead)	5000	2150
III	6 days	8000	3360	2 years (alive)	13,000	260
IV	7 days	20,800	13,000	2·5 years (dead)	6000	3240
V	—	—	—	5 years (alive)	9000	5400
VII	36 hours	14,000	11,340	3 months (alive)	8000	880

Long-Term Response

In all cases the neutrophil counts fell from their maximum post-operative values to normal values in periods ranging from three days to three weeks. In four cases (I, II, IV and V) the neutrophil counts remained in the normal range for periods ranging from two and a half to seven years. Two of these patients died; one (Case II) died after seven years from carcinoma of the lung, and the other (Case IV) after two and a half years from congestive cardiac failure. The other two patients (Cases I and V) are alive after five years. In Case V the severe lassitude present before splenectomy has been completely relieved. In two cases (Cases III and VII) the numbers of neutrophils fell to pre-splenectomy levels in 18 months and two months respectively; however, in Case III the values had fallen to below normal in one week. It will be noted (Table IV) that despite the recurrence of severe neutropenia in these cases, the total leucocyte counts were within the normal range; the increase in total leucocyte count was due to an increase in lymphocytes. None of the patients with sustained neutrophil response developed any significant infections. One patient (Case III—"failed splenectomy") has been having further infections. The leg ulcer in Case VII considerably decreased in size over a period of two months, and the infection cleared, allowing skin grafting to be performed with success after two months.

Splenic Histology

This is summarized in Table V.

DISCUSSION

INCIDENCE

There is little information about the incidence of Felty's syndrome in rheumatoid arthritis. The best figures available about the occurrence of splenomegaly and neutropenia, either singly or together, are those of Short, Bauer and Reynolds (1957), who report detailed observations on 293 patients with rheumatoid arthritis followed over a period of 20 to 25 years. They found that white cell counts lower than normal (below 5000 per cubic millimetre) were present in only five cases or 1·7% of their series; other authors they quote found leucopenia more often, in frequencies varying from 4% to 5·9%. Splenomegaly they found to be present in 6·5% of their patients, but they held that splenomegaly in association with leucopenia justified the application of the term "Felty's syndrome" to only three cases. The occurrence of splenomegaly and neutropenia in three of 293 patients indicates an incidence of about 1% of their series. The eight cases reported in this paper have been investigated over a period of ten years, six patients being seen in hospital practice and two in private consultation; all were referred because of infections or because of their abnormal blood pictures.

CLINICAL FEATURES

The classical diagnostic triad is of chronic rheumatoid arthritis, splenomegaly and neutropenia. In general, the disorder occurs in middle and older age groups, but occasionally it occurs in younger persons; thus patients aged 34 and 32 years were reported by Hutt, Richardson and Staffurth (1951) and by Rackow (1953) respectively. The arthritis has usually been present for a number of years before the clinical picture of Felty's syndrome develops; thus in our cases the duration of arthritis before the diagnosis ranged from five to 30 years, with a mean of 16 years. Perusal of the literature indicates that in general the syndrome develops in long-standing arthritis—for example, after 10 to 20 or more years; however, not uncommonly it occurs more rapidly—for example, after four to 10 years. Occasionally it presents earlier; for example, two of Felty's original patients presented after two and a half and three years and one of Steinberg's (1953)

TABLE V

Splenic Histology

	Case I	Case II	Case III	Case IV	Case VI	Case VII
Basic structure	Normal	Normal	Normal	Normal	Normal	Normal
Malpighian corpuscles	Normal	Normal	Prominent	Normal	Indistinct	Normal
Sinusoids ..	Moderate dilatation. Hyperplasia lining endothelial cells. Marked engorgement	Normal	Moderate dilatation. Moderate engorgement	Moderate dilatation. Moderate engorgement	Marked dilatation. Marked engorgement	Marked dilatation Mild hyperplasia of lining cells Marked engorgement
Pulp spaces	Moderate numbers fibroblasts, many macrophages and red cells, considerable erythrophagocytosis	Moderate number of fibroblasts and many lymphocytes	Occasional hyperplastic reticulum cells. Many lymphocytes and red cells	Few neutrophils, lymphocytes, and many red cells. No erythrophagocytosis	Few fibroblasts and plasma cells. Many neutrophils, lymphocytes and red cells	Many macrophages and lymphocytes, few neutrophils and red cells

presented after six months. Active polyarthritis may be present, or there may be residual deformity only; polyarthritis when present is often only mild.

The spleen is usually slightly to moderately enlarged, only occasionally extending below the umbilicus; rarely it is not palpable clinically, although at operation in such cases it is usually found to be enlarged (our Case II), and Steinberg, (1953). Occasionally the neutropenia precedes the development of clinical splenomegaly (Hutchison and Alexander, 1954). Moderate hepatomegaly and slight lymph-node enlargement are common. Pigmentation of the skin, although not a feature of our cases, is recorded in the literature.

Leg ulceration occurs occasionally; it was present in our Case VII, and has been recorded by Ytrehus (1946), by Peden (1949) and by Hutt *et alii* (1951). In general it has occurred with long-standing arthritis; the arthritis had been present for nine and 14 years in Peden's cases, for 12 years in our case (Case VII) and for 20 years in the case of Hutt *et alii*; however, in Smith and McCabe's case arthritis had been present for only four years. In all cases there was no obvious cause for the ulceration. The ulcers tend to be long-standing and non-healing, and not to respond to local treatment. The ulcer persisted without healing for 12 years in our case, for one year and eight months in Peden's two cases, for three years in Hutt's case, and for six months in Smith and McCabe's case. The pathogenesis of the leg ulceration is uncertain. It is possibly related to the unexplained leg ulceration which occasionally occurs with other blood dyscrasias—for example, hereditary spherocytosis (Dacie, 1960) and thrombocytopenic purpura (Witts, 1942). It should be noted that chronic leg ulceration occasionally occurs in rheumatoid arthritis uncomplicated by Felty's syndrome (Posen and Reid, 1961).

HÆMATOLOGICAL FEATURES

Peripheral Blood.—The outstanding feature in the peripheral blood is persistent, severe neutropenia, counts of less than 1000 per cubic millimetre being the rule in most established cases. However, occasional cases are recorded in which the neutrophil count rises transiently from low values to normal or near-normal values (Case II, Kanar *et alii*, 1950; Case II, Hutt *et alii*, 1951). In most cases infection, even severe infection, does not appear to increase the neutrophil count; this was a feature of six of our cases and of cases reported by Rovello (1950) and by Hutchison and Alexander (1954). However, a return to normal during pyogenic infections has been recorded (Rackow, 1953). The total leucocyte count is usually reduced, but occasionally it is normal despite the presence of severe neutropenia (Case I, Steinberg, 1953). In cases of " failed splenectomy ", however, the total leucocyte count may be normal despite fall in the neutrophil count to presplenectomy values (see discussion on splenectomy). In our cases, before splenectomy, the absolute lymphocyte counts were either reduced or normal. The absolute monocyte and eosinophil counts were normal in all our cases.

Mild normocytic normochromic or slightly hypochromic anæmia is common, but not invariable. Occasionally anæmia is severe; this, however, is usually due to some complicating or associated factor—for example, autoimmune acquired hæmolytic anæmia (our Cases I and VI), active polyarthritis, or chronic blood loss.

The platelet count is usually within normal limits, but occasionally there is mild thrombocytopenia (our Cases I, IV and V; Kanar *et alii*, 1950; Hutt *et alii*, 1951). The thrombocytopenia is usually asymptomatic and bleeding is absent.

The sedimentation rate is commonly raised (Table II); in our cases the increase was associated either with active polyarthritis or with an infective lesion, except in Case III, in which there was no obvious cause for the great increase.

Bone Marrow.—The bone marrow is usually hyperplastic (Table III; Steinberg, 1942; Hirschboeck, 1946; Hutchison and Alexander, 1954). However, sometimes the marrow is of normal cellularity (Cases III and VIII), and decreased cellularity has been recorded (Rogers and Langley, 1950; Hutt *et alii*, 1951). The increased cellularity is due primarily to granulocytic hyperplasia. Maturation arrest of the granulocytic series is often described in the literature as being present (Hutchison and Alexander, 1954). However, if the usual definition of maturation arrest is accepted—that is, a decrease of cells beyond the myelocyte stage (metamyelocytes and stab forms)—then this change appears to be uncommon. Review of the cases described in the literature as showing maturation arrest reveals that in most there was a relatively normal percentage of metamyelocytes and stab forms and a paucity only of segmented granulocytes. Only one of our patients (Case III) showed a true maturation arrest; the others showed normal proportions of developing granulocytes, with a decrease in segmented neutrophils, which numbered from zero to 2%.

Erythropoiesis may be either hyperplastic (Cases I, II, IV, VI and VII; Hirschboeck, 1946; Hutchison and Alexander, 1954) or normal; two of our patients with hyperplastic erythropoiesis had overt hæmolytic anæmia. The limited information available suggests that megakaryocytes are usually present in normal numbers, but are occasionally increased (our Case II; Kanar *et alii*, 1950).

In four cases marrow examination was performed after operation; in three (Cases I, II and IV), there was a return to normal cellularity; in Cases I and IV segmented neutrophils were present, but in Case II they were still scarce despite a normal peripheral blood leucocyte count. Case III ("failed splenectomy") showed little change.

DIFFERENTIAL DIAGNOSIS

Felty's syndrome must be differentiated from other causes of splenomegaly and of neutropenia occurring in association with arthritis. These include disseminated lupus erythematosus, and rheumatoid arthritis associated with amyloidosis, with drug-induced neutropenia and with splenomegaly due to an unrelated pathological process.

Disseminated Lupus Erythematosus

This may cause arthritis with splenomegaly and neutropenia, and should always be considered in the differential diagnosis, especially when an associated auto-immune acquired hæmolytic anæmia is present. Thus other clinical manifestations of lupus should be looked for and the L.E. cell test performed. In all but one of our cases L.E. cells were searched for, but they were persistently absent. However, one other patient was originally included in our series; she was omitted from the series when histological examination of sections of her spleen showed changes strongly suggestive of lupus.

Amyloidosis

It has been pointed out that splenomegaly occurred in 6·5% of the cases reported by Short *et alii* (1957). In the majority of cases this splenomegaly simply indicates the fact that the spleen is sharing the systemic involvement of rheumatoid arthritis. However, in occasional cases the splenomegaly may be due to amyloidosis. Missen and Taylor (1956), reviewing the post-mortem findings in a large series of cases of chronic rheumatoid arthritis, found the incidence of amyloidosis to be about 15%. Thus the possibility of amyloidosis should be considered in any case of long-standing rheumatoid arthritis and splenomegaly. In only one of our cases (Case III) was there any clinical evidence suggesting the possibility of amyloid; this patient had persistent heavy proteinuria, but there was no evidence of amyloid on histological examination of sections of her spleen. There was no histological evidence of amyloid in the spleen of any of the other patients.

Drug-Induced Neutropenia

Since patients with rheumatoid arthritis often receive numerous drugs, the possibility of drug-induced neutropenia (particularly due to phenylbutazone) must always be considered in the differential diagnosis (Fitzpatrick and Woodruff, 1955). If a patient with rheumatoid arthritis and selective neutropenia is taking a drug which may cause neutropenia, withdrawal of the drug usually results in a prompt return of the neutrophil count to normal (de Gruchy, 1958).

Splenomegaly due to an Unrelated Process

Splenomegaly may also be due to a pathological process unrelated to rheumatoid arthritis; thus Hatch (1945) reviewed 12 cases of Felty's syndrome from the literature and three of his own; he found that in at least four of these 15 cases the splenomegaly was not related to the arthritis, although one of his patients had amyloidosis of the spleen, which is now regarded as a complication of the arthritis.

TREATMENT

Adrenocortical Steroid Hormones

Review of the English literature and consideration of our cases suggests that the administration of adrenocortical steroid hormones is not a satisfactory treatment of Felty's syndrome.

Bethell, Miller and Meyers (1951) treated two patients with short courses of ACTH. The first patient, a woman, aged 47 years, was given 100 mg. of ACTH daily for 16 days; there was an increase in total leucocyte and neutrophil counts to normal values, with maximum values on the twelfth day; the maturation defect in the marrow previously present disappeared and splenomegaly subsided. However, when ACTH was discontinued, clinical and hæmatological relapse occurred within one week. The second patient, a woman, aged 55 years, was treated with 100 mg. of ACTH daily for 11 days. By the sixth day the white cell counts were normal and the spleen, which had previously been palpable, became impalpable; however, less than two weeks after cessation of ACTH therapy she returned to her pre-treatment status.

Steinberg (1953) treated a woman aged 41 years with 100 mg. of cortisone daily for seven months; the white-cell count returned to normal, and the spleen, which previously had been palpable, became impalpable; however, four weeks after cessation of cortisone therapy the white-cell count dropped and the spleen became palpable again. Steinberg treated a second patient, a woman, aged 42 years, with 20 units of ACTH given intravenously per day for 30 days; there was a moderate increase in the neutrophil count and one month after the cessation of treatment the total white-cell count was normal (the neutrophil count is not given); however, the patient apparently had a severe iron-deficiency anæmia, which was treated concurrently with iron given intravenously, and the rise in white-cell count may have been partly due to this treatment.

Hutchison and Alexander (1954) gave a woman, aged 59 years, ACTH in a daily dosage of 100 mg. for five days without any rise in the white-cell count or alteration in the bone-marrow picture.

Ellman, Cudkowicz and Elwood (1955) report two patients treated with ACTH. One patient, a woman, aged 66 years, was given a course of 900 mg. of ACTH over a period of nine days, during which time the white-cell count remained unchanged. The second patient, a woman, aged 57 years, was given 920 mg. of ACTH over a period of 13 days. This caused a temporary unsustained increase in the total white-cell count from 3000 to 6100 per cubic millimetre; the hæmoglobin value rose from 10 to 11·8 grammes per 100 ml., the number of platelets from 110,000 to 180,000 per cubic millimetre, and the percentage of reticulocytes from 2% to 5%. These levels did not persist and were reached again only after splenectomy, which was carried out to prevent renewed leucopenia after cessation of ACTH therapy.

Fitzpatrick and Woodruff (1955) report the case of a nurse, aged 43 years, given cortisone in a dosage of 100 mg. per day for one week, and then a daily dose of 50 to 75 mg. for about six months. There was an initial moderate neutrophil increase, maximum in about one month; the neutrophil count then gradually fell to pretreatment levels, despite continued cortisone treatment. These authors also mention three other cases in the literature in which steroids produced normal white-cell and differential counts with regression of splenomegaly; however, in each case leucopenia and splenomegaly recurred after cessation of therapy.

Blau and Willcox (1957) treated a woman, aged 68 years, with a course of cortisone for 15 days, starting with 300 mg. daily, the dose being gradually reduced; there was no change in the blood count, but the joint pains were relieved.

The authors of the Twelfth Rheumatism Review (1959) record two further cases in the literature in which cortisone and/or ACTH failed to improve the condition, but in which subsequent splenectomy resulted in a prompt hæmatological remission.

Three of our patients were treated with steroids. One (Case II) showed no response, and one (Case I) showed a temporary increase of neutrophils lasting eight days, with a fall to pretreatment levels despite continuous treatment. One (Case IV) had a response lasting 14 days, which was still present when the patient underwent splenectomy for an associated acquired hæmolytic anæmia.

From the limited information available, it appears that the administration of steroids may result in one of two responses—(i) no increase in neutrophils, and (ii) a temporary increase with a subsequent fall, even when treatment is continued. In general, therefore, it appears that the administration of steroids is not a satisfactory means of correcting the neutropenia of Felty's syndrome. However, it should be noted that the doses of steroids in most reported cases and in our cases were not large, and that administration was often for a short period; it is possible that larger doses may produce different results.

Splenectomy

Effect on Neutrophils.—The first splenectomy for Felty's syndrome was performed in 1932 by Hanrahan and Miller. In 1953, Steinberg reported six cases and reviewed the literature consisting of 46 cases; he did not give the total number of patients splenectomized, but noted that 37 patients had been described in those papers as "having undergone splenectomy successfully". Since Steinberg's report, at least 11 cases have been recorded in the English literature (Rackow, 1953; Hutchison and Alexander, 1954; Fitzpatrick and Woodruff, 1955; Ellman *et alii*, 1955; Blau and Willcox, 1957; Petch, 1957; Granirer, Milstein and Schmidt, 1958; Twelfth Rheumatism Review, 1959). In most of these cases follow-up investigation was short, usually being less than one year.

Short-term Response.—Review of the literature and of our own cases (Table IV) indicates that there is nearly always an immediate increase in neutrophils, which is maximal within one week. The response is

rapid, values commonly rising to normal or above-normal values within one or two days (Hutt *et alii*, 1951; Rackow, 1953; Blau and Willcox, 1957). Blau and Willcox found that counts in their patient returned to normal in six hours, and one of our patients had normal counts 30 minutes after the splenic pedicle was clamped (Figure VII). Occasionally the response is delayed for several weeks or months (Hutt *et alii*, 1951).

Long-Term Response.—Once the neutrophil count has reached its maximum, it falls to normal or near normal values, in periods ranging from several days to several weeks. In the majority of cases the count then remains in the normal range; thus in four of our six patients the counts remained normal for periods ranging

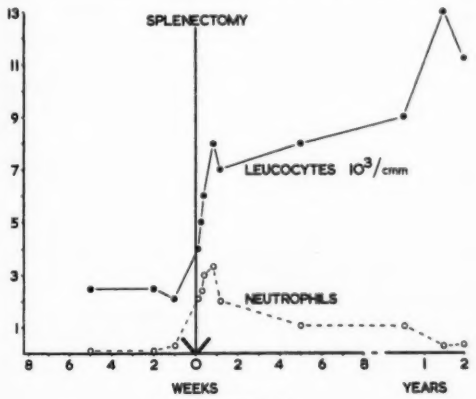

FIGURE IV

Case III, showing short-term increase in neutrophils following splenectomy, with subsequent fall to pre-splenectomy values

from two to seven years. Other long-term sustained responses recorded in the literature include five years (Hirschboeck, 1946) and 17 months (Gauld, 1949). Sometimes, however, the count falls to presplenectomy or lower than normal values, as in our Cases III and VII (Table IV and Figure IV). In general, it appears that once the neutrophil count falls to presplenectomy or less than normal values, it usually does not rise again. Nevertheless, cases have been recorded in which the number of neutrophils relapsed to presplenectomy values, but subsequently rose again to normal after some months. Thus, in Case II of Peden (1949) the neutrophil count rose after splenectomy, returned to presplenectomy values five and nine months after splenectomy, and then rose to normal at 22 months. Kanar *et alii* (1950) record a case in which there was an immediate increase to normal values after splenectomy, followed by a gradual fall to presplenectomy values two months later, and then a return to normal values five months after splenectomy.

It should be noted that in cases of "failed splenectomy" the total leucocyte count may be normal despite the fall in the neutrophil count

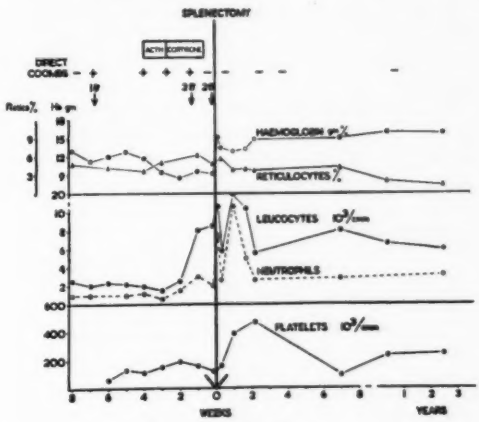

FIGURE V

Case IV, showing neutrophil response to steroids (see text) and sustained response following splenectomy

to presplenectomy values (Cases III and VII). This was due in both our cases to an increase in lymphocytes.

Effect on Anæmia and Thrombocytopenia.—Splenectomy commonly results in a sustained rise in hæmoglobin values. This was seen in three of the four cases described by Hutt *et alii*

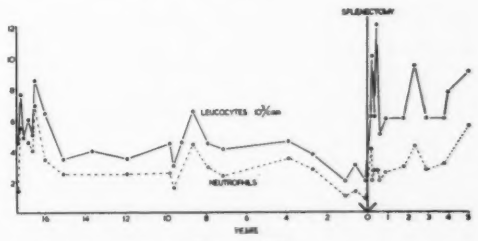

FIGURE VI

Case V, showing neutrophil counts for 16 years before diagnosis (see text), and sustained response following splenectomy

(1951) and in five of our six cases, in two of which acquired hæmolytic anæmia was present; in Case III there was a transient rise of 2 grammes per 100 ml. for five weeks, followed by relapse to presplenectomy values. Both patients with acquired hæmolytic anæmia remained in

remission, one (Case I) with a continuing remission lasting five years, and the other (Case IV) until death after two and a half years.

The limited data available suggest that thrombocytopenia, when present, is corrected and thus the platelet count remains normal—for example, our Cases I, IV and V, and the cases of Hutchison and Alexander (1954) and of Blau and Willcox (1957).

Effect on Polyarthritis.—There is little reliable information about the effect of splenectomy on the polyarthritis. Frequently active polyarthritis is absent and there is residual deformity only, as in four of our cases. There are a number of cases in the literature in which prompt relief of

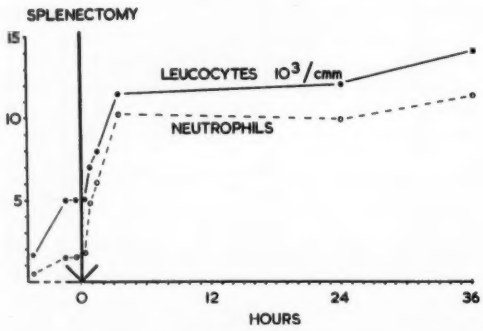

FIGURE VII

Case VII, showing immediate response to splenectomy. The first leucocyte values recorded before splenectomy represent basal ward values; the second and third are values taken after induction of anæsthesia and after laparotomy, but before ligation of splenic pedicle, respectively (see text)

the polyarthritis followed splenectomy; these include cases described by Hanrahan and Miller (1932), by Hutt *et alii* (1951) and by Steinberg (1953). Shortly after splenectomy, in our Case I the patient had a prompt remission of his polyarthritis, which has lasted five years. However, because of the variable clinical course of rheumatoid arthritis, no conclusions can be drawn about the effect on the arthritis. The editors of the Twelfth Rheumatism Review (1959) state that in cases of Felty's syndrome, splenectomy should not be considered as treatment for the arthritis.

Effect on Leg Ulceration.—The effect on the leg ulceration appears to be variable. In Smith and McCabe's (1948) case the ulceration had been present for eight months before splenectomy; after splenectomy the ulcer persisted although it decreased in size, despite the fact that the neutrophil count showed a sustained return to normal or near-lower-normal values.

Peden's (1949) first patient had ulceration present for three months; after splenectomy "the ulcer improved rapidly under wet dressing and pinch-grafting was successfully accomplished. Prior to splenectomy attempts at grafting could not be considered." Six months later two zones of ulceration had appeared within the area of pinch-grafting, and at eight months the ulcer was slightly larger than before operation. This patient's neutrophil count relapsed to presplenectomy values four months after operation. Peden's second patient had a large ulcer present for nine months before splenectomy; after splenectomy "the leg ulcer showed marked improvement, clean granulations now appeared and a split graft applied to the leg ulcer was approximately 70% successful". Four months later there was complete healing of the leg ulcer, which had remained healed at the time of the last follow-up investigation 22 months after splenectomy; at this time the neutrophil count was normal.

In Case I of Kanar *et alii* (1950) the patient had pretibial ulcers on both legs, present for two months before splenectomy; after splenectomy the smaller ulcer on the right leg healed completely and the larger on the left leg healed slowly after pinch-grafting; neutrophil counts were normal after splenectomy.

Hutt *et alii* (1951) describe a case in which a small ulcer was present on the left leg for three years; after following splenectomy it healed; nine months after operation it was still healed and the neutrophil count was normal.

Our patient's leg ulcer showed immediate improvement, with clearing of infection and the appearance of healthy granulations; grafting was performed two months after splenectomy, and it is now fully healed three months after splenectomy; the neutrophil count, however, has returned to near presplenectomy values.

Histological Findings in the Spleen.—The histological findings are reviewed by Hutt *et alii* (1951). They have found that the spleen maintains its normal basic structure; the sinusoids are dilated and the lining endothelial cells show evidence of hyperplasia; the pulp sometimes shows an increase of granulocytes and lymphocytes, and in some cases numerous eosinophils. The Malpighian corpuscles in their cases varied, being normal or large in three cases and atrophied in one. In general, our six cases showed similar changes, but all changes were not present constantly (Table V). Thus, although the sinusoids were dilated and engorged in all but one case, in only two cases was there

hyperplasia of their lining cells. The pulp showed an increased number of lymphocytes and less often of neutrophils, but in no case was there an excess of eosinophils. A moderate increase in fibroblasts was present in two cases; macrophages were increased in three cases. In Case I, in which associated hæmolytic anæmia was present, erythrophagocytosis occurred.

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HOLMES–ADIE SYNDROME WITH PROGRESSIVE AUTONOMIC DEGENERATION[1]

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SUMMARY

A case is described of coexisting autonomic degeneration and Holmes-Adie syndrome. Physiological investigations suggest that both conditions are due to post-ganglionic degeneration of the autonomic nervous system. A common pathogenesis is postulated.

THE syndrome of tonic pupil associated with absence of tendon jerks is an uncommon condition usually discovered as an incidental finding and causing no discomfort to the patient (Holmes, 1931; Adie, 1931, 1932). The pupillary reflex changes have been ascribed to a lesion in the ciliary ganglion (Adler and Scheie, 1940; Russell, 1958; Cameron, 1959), but Haas (1959) postulates a hypothalamic site to explain the association of pupillary and tendon-reflex disturbances.

Reports of more extensive autonomic involvement than the pupillary reflexes are rare. Croll and Duthie (1953) described a patient with the syndrome who also exhibited postural hypotension due to failure of autonomic vasomotor reflexes, and Ross (1958) reported an example of the syndrome in which there was evidence of generalized sudomotor loss, but without impairment of vasomotor reflexes. Ross attributed this association of generalized autonomic dysfunction with a tonic pupil to a "rare coincidence".

The present description is of a third case of Holmes-Adie syndrome in which there is evidence of generalized autonomic degeneration, both sudomotor and vasomotor reflexes being impaired. Investigations carried out in an attempt to determine the site of the lesion are described.

CASE REPORT

A motor mechanic, aged 32 years, presented on March 15, 1960. He gave a twelve years' history of increasing fatigue in hot weather, associated with an abnormality of sweating. He had never been conscious of sweating in the face and arms, and at first it appeared that the left leg sweated excessively on exertion. Sweating in the right leg became progressively less and finally disappeared, and seven years earlier sweating in the left leg had stopped also. Sweating of the trunk had persisted and was excessive in hot weather. However, this would diminish after a few hours of work, and it was then that he would become exhausted and unable to continue physical activity. He found that by taking a hot shower he could induce sweating of the trunk, and was thereby enabled to resume work for half an hour, after which sweating would stop again. He could obtain relief from his state of exhaustion by taking a cold shower or by sitting with the feet in cold water. He noted that he drank excessively in hot weather and passed large amounts of pale urine. He volunteered that by deliberately breathing through his mouth he felt better in hot weather.

His vision remained unimpaired; the sense of balance was unaffected; erection, emission, micturition and bowel function were all normal. The patient denied the possibility of any contact with venereal disease. Past illnesses included mumps, measles, scarlet fever, infective hepatitis and appendicitis, but none of these were related in time to the onset of the sweating loss. He had suffered from mild asthma until 10 years previously, when the disease was relieved by discarding a feather pillow. His mother was thought to have contracted poliomyelitis late in the pregnancy, and as a result of this was left with palsy of the left arm and leg.

On examination, the patient was of stocky build, 1·66 metres tall, weighing 70·8 kg. When he was lying at rest in a room temperature of 24° C., the pulse rate was 90 per minute, the oral temperature was 36·9° C., and the blood pressure was 125/85 mm Hg. The mouth was moist. The skin of the limbs was dry and coarse, while that of the forehead, chest, axillæ and abdomen was moist. The left pupil was oval in shape and slightly larger than the right. Neither pupil reacted to direct intense light, but on accommodation both constricted slowly to pin-points, returning even more slowly over 45 sec. to their former size. Eye movements were normal, and there was no nystagmus. Vision in both eyes was 6/6, J1. On changing vision from infinity to the Jaeger chart, the patient had no difficulty in immediately reading the J1 paragraph. The heart, lungs, abdomen and external genitalia were normal to clinical examination. The urine was free of protein and sugar. The biceps, triceps, supinator, knee and ankle jerks were all absent. Knee and ankle jerks were also tested during the Jendrassik reinforcement manœuvre, but could not be elicited. The jaw jerk was active. There was no impairment of motor power or of any sensory modality including that of vibration, and there was no incoordination of voluntary movement. Rhombergism was not present. The patient seemed of normal

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intellect. The blood Wassermann reaction was negative. The cerebro-spinal fluid was not examined.

A year after the original examination there had not been any extension of the area of sweat loss or change in symptoms. However, it was noted that the left pupil was now slightly smaller than the right. It remains to be seen whether this man will develop the impotence and postural hypotension characteristic of advanced cases of the disease.

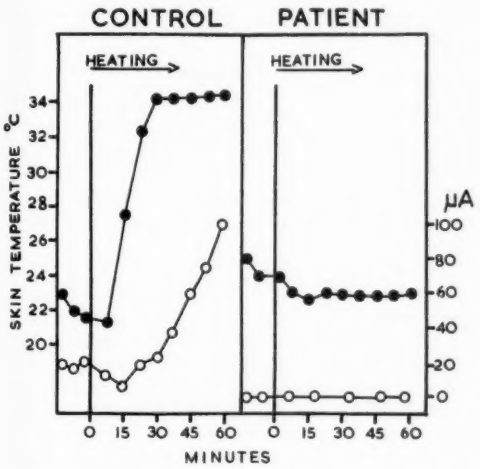

FIGURE I

The effect on skin temperature and current flow through the tip of the fifth finger during indirect body heating. Solid circles, skin temperature in degrees Centigrade. Open circles, current flow (μA). Current flow is inversely related to skin resistance

Physiological Data

Adler-Scheie Test.—The instillation of 2·5% "Methacholine" (acetyl-beta-methyl choline chloride, Light & Co. Ltd.) in distilled water into the conjunctival sac of both eyes produced constriction of both pupils which came on after 10 minutes, was fully developed at 30 minutes and had not completely reverted to normal after one hour. The resting diameter of the left pupil was 3 mm. and of the right 4 mm.; 30 minutes after "Methacholine" had been instilled the diameters were 1·5 and 2 mm. respectively. In all, six drops of "Methacholine" were given into each eye, two drops at intervals of two minutes. Adler and Scheie (1940) showed that this test was diagnostic of Adie's pupil, because neither the normal nor an Argyll-Robertson pupil constricts to this concentration of "Methacholine". This test is thought to reflect increased sensitivity of the partly denervated pupil to cholinergic agents.

Indirect Heating.—The patient was seated comfortably with his legs in a stirred water bath at 44° C. The body was covered by blankets, but the arms were uncovered and rested on a table. The room temperature was controlled at 25°±1° C. throughout. Skin temperature and resistance were measured at three points on each hand and one on each forearm before and during heating. After one hour, the mouth temperature had risen to 38·4° C., and sweating occurred over the face, chest, abdomen and back; but there was no change in skin temperature or resistance on the hands and forearms. This absent response is shown in comparison with that of a normal subject in Figure I, and indicates interruption of the vasomotor and sudomotor fibres to the points tested.

Hand Blood Flow.—Bilateral hand blood flow was measured by venous occlusion plethysmography, with the use of water-filled, temperature-controlled plethysmographs (Greenfield, 1954). There were no spontaneous variations in hand blood flow, nor was there any vasoconstriction during such stimuli as a loud noise or the application of ice to the forehead and neck (Figure II). These procedures consistently produce marked vasoconstriction in hands with normal sympathetic supply. This finding provides further evidence of the loss of vasomotor innervation of the hands.

Heat Flow after Ulnar Nerve Block.—The patient lay at rest on a couch in a thermostatically controlled room at 23° C. in which the air was gently circulated by fans. Heat flow from the pulps of both little fingers was measured simultaneously with copper-tellurium heat-flow discs (Hatfield, 1950). After a period of control measurements, the left ulnar nerve was blocked at the elbow with 2% lignocaine solution. There was no alteration in heat flow from the blocked side compared with the other side, even when anæsthesia of the left finger skin was complete. In normal subjects, ulnar nerve block increases heat flow from the skin of the finger by interrupting the resting vasoconstrictor tone.

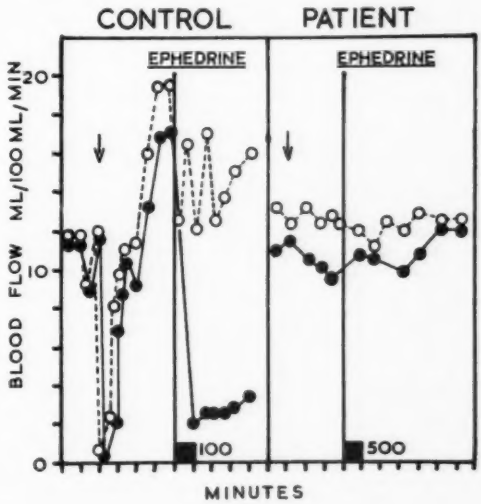

FIGURE II

Hand blood flow after reflex sympathetic stimulation and the intraarterial infusion of ephedrine. At the arrow, ice was placed on the side of the neck. Solid circles, infused hand; open circles, control hand. The solid square represents the period of infusion of ephedrine, and the adjacent figures are the doses in microgrammes per minute

Intraarterial Drug Infusion.—A 23 gauge short-bevel needle was inserted into the brachial artery at the elbow and connected via "Polythene" tubing to a constant infusion machine. In control periods, saline (0·9% w/v) was infused at the rate of 4 ml. per minute, and this was replaced as required by the drug made up so that the dose per minute was contained in 4 ml.

Hand blood flow was measured bilaterally by plethysmography. Injection of acetylcholine chloride (80 μg per minute) caused an increase in hand flow and demonstrated that drugs infused in this manner were reaching the hand blood vessels.

Methylamphetamine hydrochloride (Burroughs Wellcome) and ephedrine hydrochloride (Elliott) in doses of 500 and 1000 μg. over one minute, did not produce any alteration in hand blood flow. In normal subjects doses as small as 50 μg. for one minute cause constriction of hand blood vessels lasting a few minutes, while doses as large as 1000 μg. produce profound vasoconstriction lasting at least one hour (Parks, Sandison, Skinner and Whelan, 1961). Figure II illustrates this absence of response to 500 μg. of ephedrine compared with the response to 100 μg. in a normal hand.

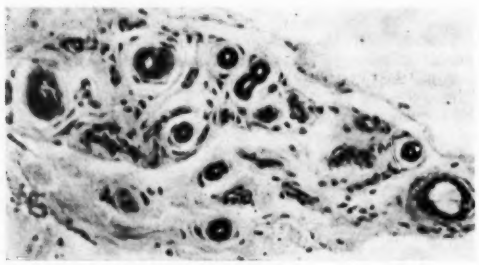

FIGURE III

Sweat glands and ducts from the pulp of the fifth finger

Recent findings suggest that ephedrine and "Methedrine" act by releasing noradrenaline from its site of storage in the post-ganglionic fibre (Burn and Rand, 1958; Axelrod and Tomchick, 1960). The absence of response to these drugs in high concentration indicates that the post-ganglionic mechanism has lost its store of catecholamine. This absence of response of the hand blood vessels to ephedrine and "Methedrine" has also been demonstrated in patients after stellate ganglionectomy (Parks *et alii*, 1961).

Methacholine Given Intradermally.—The injection of "Methacholine" (500 μg.) into the skin of the forearm did not produce any local sweating or drop in electrical skin resistance. In a control normal subject this dose of "Methacholine" produced marked local sweating at and around the site of injection with a precipitous fall in skin resistance. Intradermal saline injection had no effect in either the patient or the control.

The absence of a sudomotor response to methacholine suggests either that the sweat glands have atrophied, or that, if present, they are no longer capable of forming sweat.

Skin Biopsy.—Skin was taken from the pulp of the left little finger with a 3 mm. punch after ulnar-nerve block at the elbow. This area was chosen since it had been conclusively shown to be sympathetically denervated. Skin was also taken from the same situation in two healthy male subjects. The specimens were fixed in formol-saline, and serial sections were stained with hæmatoxylin and eosin. The sweat glands and ducts from the patient (Figure III) did not differ histologically from normal. There was no evidence of atrophy, the glandular appearance being consistent with a resting stage of secretion. This finding of structurally normal glands which are nevertheless refractory to sudorific drugs has been discussed by Randall and Kimura (1955) and by Ross (1958). The observation does not assist in the localization of the neurological lesion.

Cardio-Vascular Reflexes.—While the patient was lying at rest, the blood pressure by sphygmomanometry was 130/90 mm Hg. The pulse rate as recorded by electrocardiography varied with respiration, and on the patient's standing there was immediate tachycardia which settled to a steady level in 45 sec., while the blood pressure remained at 130/95 mm. Hg. This response to posture indicated that the loss of peripheral vasomotor control was not sufficient to affect postural adjustments and did not involve the cardiac reflexes.

Pressure over the left carotid sinus reduced the pulse rate from an average resting value of 90 per minute to 62 per minute. This response was consistently reproducible on the left, but on the right no change in heart rate was obtained. Since a carotid sinus reflex is frequently difficult to demonstrate in normal subjects, the absence of response on the right side was not considered important.

These findings suggested that there was no gross abnormality in the autonomic innervation of the heart.

DISCUSSION

The symptoms of fatigue and discomfort in hot weather complained of by this patient were a consequence of his impaired heat balance due to loss of sudomotor reflexes in all four limbs. This loss had been gradual over 12 years, but during the 12 months of observation appeared not to have progressed further. It has been shown that while post-ganglionic fibres remain intact, the sweat glands continue to respond to cholinergic drugs (List and Peet, 1938; Hyndman and Wolkin, 1941; Gurney and Bunnell, 1942; Netsky, 1948), but that after post-ganglionic section the response soon disappears (Hyndman and Wolkin, 1941; Janowitz and Grossman, 1950). The absence of a local sweating response to injection of "Methacholine" in this patient suggested either that the post-ganglionic cholinergic fibres supplying the sweat glands had degenerated, or that the glands themselves were primarily affected. The former possibility seems the more likely, in view of the histologically normal appearance of the glands in biopsy and of the evidence for an associated post-ganglionic lesion of noradrenergic vasomotor fibres to the same regions. Thus ulnar-nerve block did not increase heat flow from the little finger, vasomotor responses to sensory stimuli and to indirect heating were absent, and the failure of the vessels to respond to sympathomimetic agents, which act by release of noradrenaline from the post-ganglionic mechanism, indicated post-ganglionic autonomic degeneration. Unrelated defects in both cholinergic and noradrenergic transmission localized to specific areas would appear to be unlikely. The findings indicate a ganglionic or post-ganglionic lesion, and while the existence of a central lesion affecting the autonomic centres cannot be excluded, it need not be invoked to explain the autonomic effect.

Adler and Scheie (1940) and Russell (1958) concluded that the lesion in Holmes-Adie syndrome was post-ganglionic, involving the ciliary ganglion, and Cameron (1959) described cases of herpes ophthalmicus with tonic pupils, suggesting also that the site of the disturbance lay in the ciliary ganglion. Since these findings indicate lesions of identical anatomical positions in the innervation both of the eye and of the limbs, it is possible that the pupillary involvement and the sudomotor and vasomotor loss may have a common ætiology and be a manifestation of the same generalized autonomic dysfunction. This likelihood was suggested by Croll and Duthie (1935) when describing a patient with Holmes-Adie syndrome who also suffered from postural hypotension. However, Ross (1958) considered that the association with sudomotor loss in his patient was coincidental.

Holmes-Adie syndrome and autonomic degeneration are both rare conditions, and if they are separate entities their occurrence together in the same patient would be very rare indeed. Only three cases have been reported, but this infrequency cannot be taken as indicating the true incidences of this association, since the slowly progressive nature of autonomic degeneration may not produce symptoms for many years, if at all. Unless vasomotor reflexes become involved to an extent resulting in postural hypotension, the condition may pass unnoticed, particularly in cool climates where a reduction in sweating would not be critical to comfort. Indeed, the present patient was symptomless during winter. These considerations make it plausible to consider the tonic pupil and sudomotor and vasomotor loss as a manifestation of a common disorder of the peripheral autonomic system, but only a careful follow-up of patients with tonic pupil over a period of years with the use of sensitive tests of vasomotor and sudomotor function could establish this hypothesis.

The cause of absence of tendon reflexes in Holmes-Adie syndrome remains unexplained. Only three of Adie's original five cases and 19 of 54 cases described by Holmes (1931) demonstrated this relationship. Of 44 patients examined by ophthalmologists, only nine were considered to have absence of reflexes, but neurologists have reported groups of cases in which the reflexes were abnormal in every case (Adie, 1932). However, Hufschmidt and Schaltenbrand (1959) claim to have demonstrated a phasic stretch reflex in most cases of constitutional areflexia, including Holmes-Adie syndrome, when tested electromyographically. Areflexia is therefore not a consistent finding in patients with atonic pupils, and it is thus not possible to be certain that it is related to the pathogenesis of the pupillary changes.

ACKNOWLEDGEMENTS

We are indebted to Dr. G. A. Hunter for examining the biopsy specimen, and to the patient for cooperating throughout the study.

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FAMILIAL ENDOCRINE ADENOMATOSIS WITH ULCEROGENIC TUMOUR OF THE PANCREAS[1]

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SUMMARY

A further instance of the syndrome of familial multiple endocrine adenomatosis is described, an unusual feature being the occurrence in two generations of the less common non-beta-cell adenoma of the pancreatic islets, causing in the daughter typical clinical manifestations of the Zollinger-Ellison syndrome—primary jejunal ulceration and recurrent marginal ulceration, requiring total gastrectomy and pancreatectomy. This patient has also a pituitary tumour, the evidence pointing to a chromophobe adenoma, and may possibly have parathyroid hyperplasia or adenoma. The father of the patient was shown at autopsy to have parathyroid adenomas, a pituitary chromophobe adenoma, adrenal cortical hyperplasia and a pancreatic islet-cell adenoma morphologically resembling most closely that seen in his surviving issue.

OCCASIONALLY the report of a bizarre clinical syndrome may set off a chain reaction resulting in discoveries of profound scientific interest. At about the time when the biochemical significance of the carcinoid syndrome came under active investigation, Zollinger and Ellison (1955) described four cases, and reviewed four others previously reported, of primary ulceration of the jejunum associated with tumours of the pancreatic islets. A humoral ulcerogenic substance, the existence of which had previously been postulated by Poth, Manhoff and de Loach (1948), was implicated. Clearly this was not insulin, for patients with what has subsequently come to be referred to as the Zollinger-Ellison syndrome display no hypoglycæmic propensities; nor were Eiseman and Maynard (1956) able to demonstrate the presence of a hyperglycæmic factor (glucagon) in the serum of two patients with the Zollinger-Ellison syndrome. Recently it has been demonstrated (Gregory, Tracy, French and Sircus, 1960) that a substance with properties similar to gastrin may be isolated from islet tumours in the Zollinger-Ellison syndrome. This is a discovery of considerable importance, for there are on record case reports in which peptic ulceration has been associated with hyperplasia only of the islets (Zollinger and McPherson 1958; Summerskill, 1959). Production of a gastrin-like hormone stimulating gastric secretion may therefore be a normal function of pancreatic islets, an item of information which may prove to be relevant to the problem of the pathogenesis of duodenal ulceration.

The Zollinger-Ellison syndrome is thus of great interest to gastroenterologists. In the 40 or so cases reported since 1955 (Ellison, 1956; MacKenzie and Norvell, 1960), progressive peptic ulceration with its complications has been the most common presenting clinical feature. The ulcers are commonly multiple, and in over 50% of cases occur at sites beyond the first part of the duodenum. Indeed it is most likely, as in the case reported here, that most cases of primary peptic ulceration of the small intestine (review of Evert, Black and Dockerty, 1948) described prior to 1955 were examples of the Zollinger-Ellison syndrome. Death has occurred in more than half the reported cases from the complications of perforation or hæmorrhage, often from marginal ulcers occurring after first and second subtotal gastrectomies. In all cases studied in detail, profuse gastric hypersecretion (up to six to 10 litres of acid secretion produced daily) has been demonstrated; and in some instances giant hypertrophy of the gastric mucosa (Menetrier's syndrome) has been demonstrable gastroscopically and/or radiologically (Zubrod *et alii*, 1958).

More recently, from reports of Maynard and Point (1958), Summerskill (1959), Priest and Alexander (1957) and Verner and Morrisson (1958), it has become apparent that patients with islet adenomas may present with symptoms of wasting together with fatty or watery

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diarrhœa, the latter causing severe hypokalæmia. It is presumed that the intense outpouring of gastric juice causes significant lowering of pH of contents of the small gut, inhibiting fat digestion.

The Zollinger-Ellison syndrome is of considerable interest also to the endocrinologist. In 1953 Underdahl, Woolner and Black described several cases of multiple endocrine adenomas.

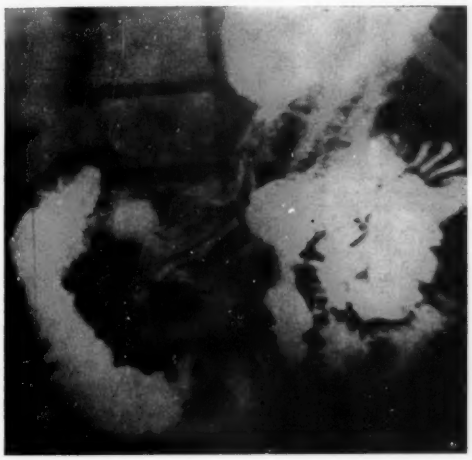

FIGURE IA

Barium meal X-ray film, 1952. Note irregularity of the mucosal pattern in the proximal part of the jejunum

The link between endocrinology and the gut had been forged by Cunningham, Howe and Evans in the previous year; their patient had adrenocortical adenomas and both beta-cell and malignant non-beta-cell adenomas, together with jejunal ulceration and gastric hypersecretion. In five of the 24 cases of the Zollinger-Ellison syndrome reviewed by Ellison in 1956, other endocrine adenomas (pituitary, parathyroid and/or adrenal cortex) were present. There is, to add to the theoretical interest, evidence that the syndrome of endocrine adenomatosis is an inherited disorder (Wermer, 1954).

The case described here in some detail has been previously reported as an example of primary ulceration of the jejunum (Levitt and Saint, 1955). That the patient has subsequently been shown to have the Zollinger-Ellison syndrome is perhaps only of parochial interest; of greater significance is the documentation of inheritance of the syndrome of multiple endocrine adenomatosis. Both the patient and her father are shown to have multiple adenomas.

Of subsidiary interest in the case are aspects of applied physiology which cast faint light on the genesis of peptic ulcer, and on the site of intrinsic factor production in the human stomach.

CLINICAL RECORD

The patient is now married and aged 31 years. Between the ages of 19 and 23 years she was admitted to hospital on five occasions with recurrent melæna, each requiring transfusion of one to two litres of blood. She complained of persistent epigastric pain occurring two to three hours after meals and relieved by alkalis. She was submitted to three barium-meal X-ray examinations. On each occasion the stomach and duodenal bulb were reported on as normal; but on retrospective viewing gross irregularity of the mucosa of the third part of the duodenum and jejunum is visible (Figure IA).

In October, 1953, at the age of 24 years, she was admitted to hospital because of acute upper abdominal pain. The first of a series of explorations revealed that perforation of one of six peptic ulcers present in the first 30 cm. of the jejunum had occurred. This

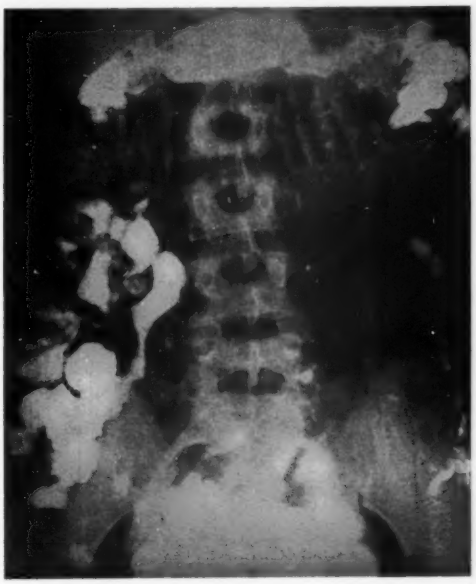

FIGURE IB

Barium follow-through X-ray examination, showing clumping of barium in small intestine

was oversewn. Closer questioning at this time brought to light the existence of three further relevant symptoms. She had never menstruated; the gynæcological opinion given was that she had an infantile uterus. She had been subject, in addition, to ulcer pain and to attacks of diarrhœa, and her stools were constantly greasy. Also, she had lost a considerable amount of weight. Overnight gastric juice secretion (12 hours) measured 3 litres; the HCl concentration was 120 mEq/l. A spot fæcal fat estimation showed

32% of fat. Some clumping and segmentation of barium were seen in the ileum in follow-through film studies (Figure IB).

In December, 1953, she was submitted to an elective subtotal gastrectomy (Pólya) with resection of 40 cm. of the ulcer-bearing area of the jejunum. A warning

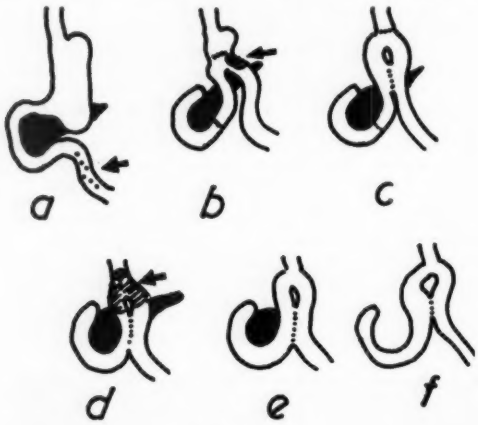

FIGURE II

Diagram showing site of ulcers (arrows) and surgical procedures undertaken: (*a*) perforation of primary jejunal ulcer—oversewn, October, 1953; (*b*) Pólya gastrectomy, December, 1953, followed by marginal ulceration with perforation, January, 1954; (*c*) "total" gastrectomy, January, 1954; (*d*) œsophago-jejunal ulceration, 1959–1960; (*e*) œsophago-jejunectomy with subtotal pancreatectomy, March, 1960; (*f*) completion of total pancreatectomy

note of troubles to come was sounded when it was found that the HCl concentration of the post-operative gastric aspirate was 80 mEq/l. Four weeks after operation she returned to hospital with upper abdominal pain and fever, followed by melæna. Exploration

TABLE I

Serum Vitamin B_{12} Levels[1]

Date	Total Vitamin B_{12} (μμg/ml)	Free Vitamin B_{12} (μμg/ml)	Comment
31.7.56	562	20	2½ years after "total" gastrectomy
7.1.57	846	20	3 years after "total" gastrectomy
26.8.59	451	20	4½ years after "total" gastrectomy
16.5.60	562	53	2 months after "true" gastrectomy
3.3.61	651	20	1 year after "true" gastrectomy. Vitamin B_{12} given

[1] Normal ranges: Total vitamin B_{12}, 190 to 875 μμg per millilitre; mean, 456 μμg per millilitre. Free vitamin B_{12}, 20 to 270 μμg/0; mean, 52 μμg/9 (Nicholas and Pitney, 1958).

(her third laparotomy) revealed perforation of a stomal ulcer. She was then submitted to a supposed total gastrectomy with the construction of a side-to-side jejuno-jejunostomy. The post-operative course was stormy, characterized by pulmonary collapse, hypokalæmia and intraabdominal abscess formation requiring further exploration and drainage.

In the ensuing five years (1954 to 1959) she enjoyed remarkably good health. She was able to eat surprisingly large meals without post-prandial distress, and her weight increased from 100 to 130 lb. She married and became involved in a variety of social activities. The level of hæmoglobin was watched with interest; it remained normal (she was taking iron preparations

TABLE II

Observations on Intestinal Absorption

——	Before Pan-createctomy	After Pan-createctomy	Normal Range
Fæcal fat (per cent.) ..	—	52, 59	<25
Serum carotene content (μ per 100 ml.) ..	47, 30	7	>70
Xylose absorption (grammes in 5 hours in urine) ..	3·3	0·7	4·1 to 8·2
Serum cholesterol content (milligrammes per 100 ml.)	293	134	200 to 250
Serum albumin content (grammes per 100 ml.)	3·8	2·5	—

by mouth), and after four years it was the cause of surprise that macrocytes had not appeared in blood smears. Whilst she was on holiday in London in 1958, Dr. Mollin of Hammersmith Hospital demonstrated for our interest that the serum vitamin B_{12} level was normal and that the patient was capable of absorbing B_{12}-intrinsic factor complex normally.

FIGURE III

Lateral X-ray view of skull showing widening and deepening of the sella turcica, and erosion of the posterior clinoid process

In March, 1959, she complained once more of pain in the left upper abdominal quadrant radiating to the back; occasionally she felt a burning retrosternal sensation after drinking milk. Also she began to suffer from periodic frontal headache. Examination of the abdomen and the optic fundi was unrevealing. The hæmatological findings were normal in all respects, and a gastric biopsy indicated that gastric mucosa was in fact present. Mild absorptive deficiencies were demonstrated biochemically, and an X-ray examination of the skull showed evidence of enlargement of the sella turcica.

A year later (February, 1960) her condition had greatly deteriorated. Her appetite was poor, she had lost much weight, and she complained of constant pain in the left upper quadrant of the abdomen, where an ill-defined mass could be palpated. The hæmoglobin value had fallen to 10·4 grammes per

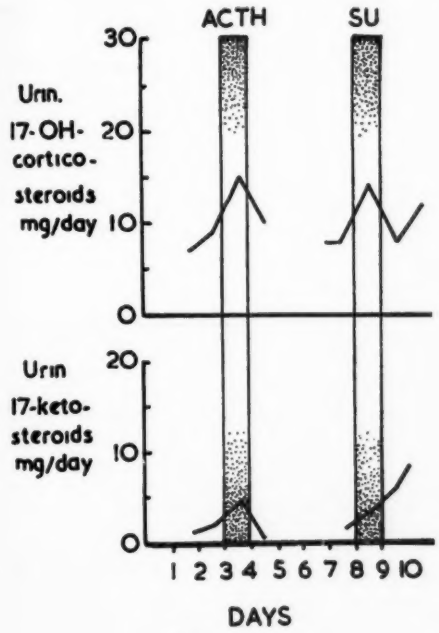

FIGURE IV

Urinary 17-ketosteroid and 17-OH steroid excretion following administration of (*a*) ACTH and (*b*) SU-4885

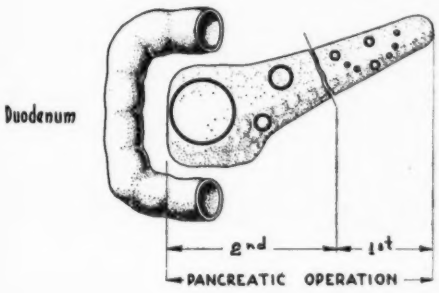

FIGURE V

Site of macroscopic pancreatic adenomas

100 ml.; the white cell count was 15,000 per cubic millimetre, and the erythrocyte sedimentation rate was 112 mm. in one hour (Westergren). A confident diagnosis of pancreatic adenomatosis could now be made, and the pre-operative opinion was that invasive malignant change had occurred. In the event (her fourth major surgical procedure) she was found to have a large peptic ulcer measuring three by two centimetres situated at the junction of the œsophagus and jejunum and adherent to the body of the pancreas. A rim of recognizable gastric mucosa 1·2 cm. in width had been left behind at the previous "total" gastrectomy. The resection of the lower part of the œsophagus, the ulcerated jejunum and the body and tail of the pancreas, with reconstitution of the œsophago-jejunal anastomosis, proved to be a formidably difficult undertaking. Several small adenomas were visible in the resected pancreas.

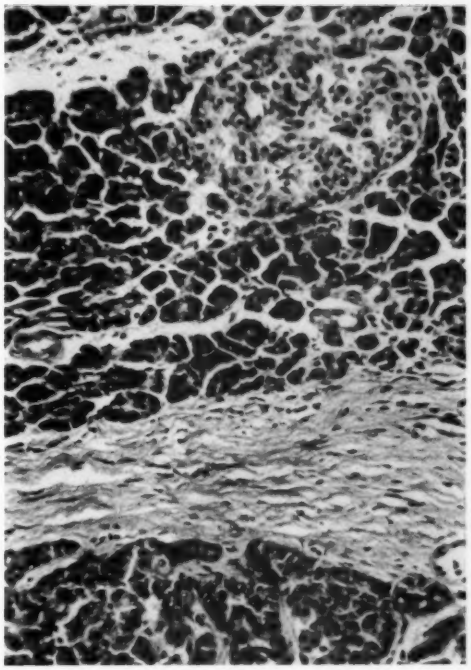

FIGURE VIA

Body and tail of patient's pancreas (×140): normal islet at top. A fibrous capsule separates a visible nodule below; in this nodule islet cells are obviously larger

The post-operative course was cyclonic. The patient required further surgical intervention to relieve an early high intestinal obstruction, and an abscess cavity in the splenic bed was drained at the same time. From this she developed a fistulous tract connected, it was believed, to the jejunum and the pancreatic stump. Further subacute obstruction and lung infection with empyema were hurdles successfully jumped.

The patient now required treatment with vitamin B_{12} given intramuscularly, with iron given by injection, and with pancreatic extract given orally. Carbohydrate tolerance was normal. She was readmitted to hospital in August, 1960, for elective removal of the head of the pancreas. At this final abdominal exploration an ovoid tumour was located in the head of the pancreas which, with the remainder of the

body, was excised. On this occasion the post-operative course was uncomplicated. Glycosuria developed: her insulin requirement has proved to be 16 units per day.

At present she is barely maintaining her weight on a protein-rich diet, with pancreatic extract, vitamin B_{12},

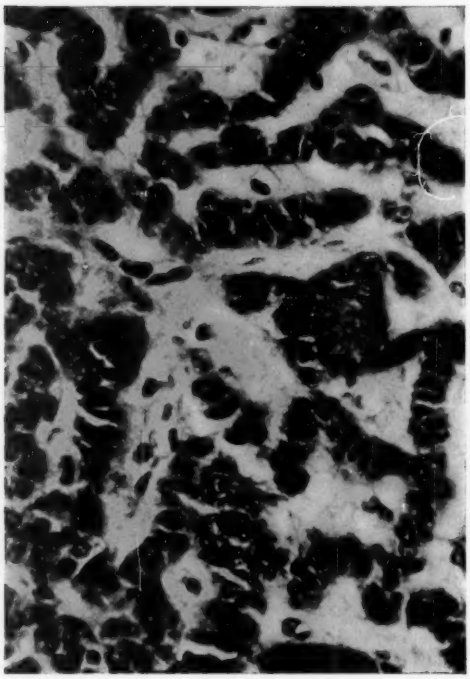

FIGURE VIB

Tail and body of patient's pancreas (×350); anastomosing cords and strands of islet cells in a macroscopically visible tumour. (Compare Figure VIIB)

iron and insulin. She still complains of periodic headache. However, the visual fields are normal, and we are inclined to postpone for a while any surgical procedure in the region of the pituitary fossa.

The Patient's Family

The patient is an only daughter and has not, of course, produced issue. Her mother has enjoyed excellent health. Nothing is known about the health of her grandparents. In 1955 her father, at the age of 61 years, was admitted to the Repatriation Hospital with symptoms and signs of advanced renal insufficiency, from which he died shortly afterwards. He is not known to have experienced symptoms or complications of peptic ulcer. We are indebted to Dr. Beryl Lawrence, Pathologist to the Repatriation Hospital, Hollywood, for an account of the autopsy findings. In brief, the kidneys were contracted and showed fibrotic changes with hyalinization of the glomeruli. All four parathyroids were visibly enlarged. A large single tumour was present in the tail and body of the pancreas, and the pituitary also was thought to be enlarged. The adrenals were of normal appearance. The stomach and duodenum were normal. The microscopic findings are presented in detail below (see "Pathological Studies").

Gastro-Enterological and Endocrine Studies

The physiological abnormalities (gastric hyperacidity and steatorrhœa) present prior to 1954 have been commented upon above.

Serum levels of vitamin B_{12} were estimated on several occasions and found to be normal (Table I).

A gastric biopsy performed a year prior to her final gastrectomy showed normal gastric mucosa. It was estimated later that an area of approximately 10 sq. cm. of gastric mucosa adjacent to the œsophago-gastric junction had been left at the "total" gastrectomy of 1954. From this small area of mucosa sufficient intrinsic factor had emanated to permit normal vitamin B_{12} absorption, and enough gastric juice to cause extensive and penetrating ulceration of the œsopgaho-gastro-jejunal mucosa.

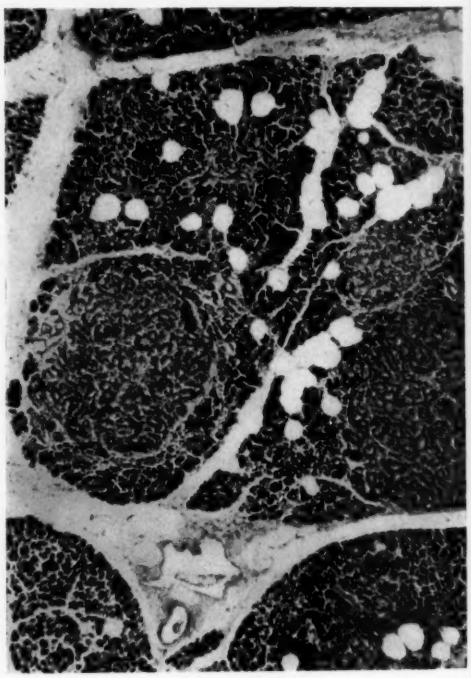

FIGURE VIC

Body and tail of patient's pancreas (×35). The three groups of hyperplastic islet tissue seen in the centre show the widespread adenomatosis found on microscopic examination

Since pancreatectomy there has been evidence (despite the administration of pancreatic enzymes) of severe intestinal malabsorption (Table II).

The transit time through the small intestine of barium mixed with porridge is normal, opaque material reaching the caecum in six hours.

It is worthy of note that since total pancreatectomy the serum amylase levels still remain at 40 to 64 Somogyi units.

The X-ray film of the skull (Figure III) shows enlargement and deepening of the sella turcica, with thinning of the dorsum sellæ and posterior clinoids. Evidence that this is due to the presence of a chromophobe adenoma is adduced from endocrinological investigations. Urinary gonadotrophin excretion is persistently less than 5 mouse units (normal 5 to 75 units). Serum protein-bound iodine levels have been consistently in the low-normal range (3·8 μg per 100 ml.). However, uptake of ^{131}I by the thyroid gland after the administration of thyrotropic hormone shows a normal increase. Urinary neutral 17-ketosteroid excretion is low (1·3 to 3·0 mg. in 24 hours); 17-OH steroid excretion is normal (4·8 to 8·7 mg. in 24 hours). Both 17-ketosteroid and 17-OH steroid excretion increase substantially in response to ACTH infusion, and after the administration of SU-4885 (method of Liddle *et alii*, 1959), an inhibitor of 11-hydroxylation of steroids which may be used to test

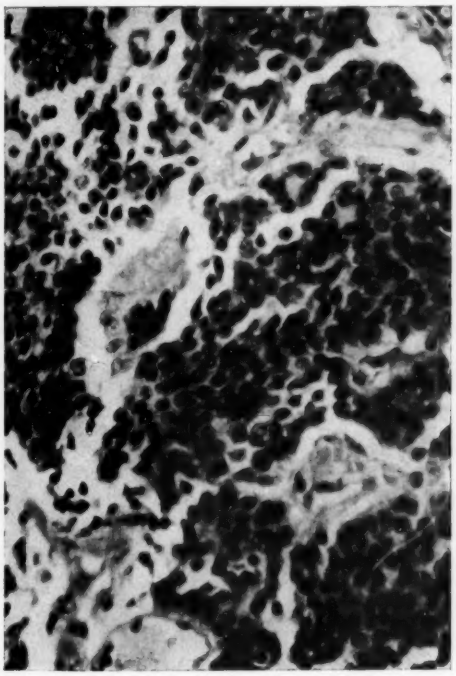

FIGURE VID

Patient's large pancreatic tumour (×350); clumps of uniform cells separated by connective tissue. (Compare Figure VIIA)

pituitary reserve. The response shown in Figure IV is interpreted as showing normal capacity of the pituitary to release ACTH.

Evidence of hyperparathyroid activity is equivocal. Serum calcium levels have ranged between 8·9 and 11·8 mg. per 100 ml. Levels of phosphate in the serum are consistently low (mean, 2·7 grammes per 100 ml.). Urinary calcium excretion on a low calcium intake is 7·4 mEq in 24 hours (normal, 5 to 15 mEq); urinary phosphate excretion is 35 mEq (normal, 30 to 90 mEq in 24 hours). However, X-ray examination of the hands shows no cortical erosion of the phalanges, and there is no evidence of renal acidosis, calcinosis, or calculus formation.

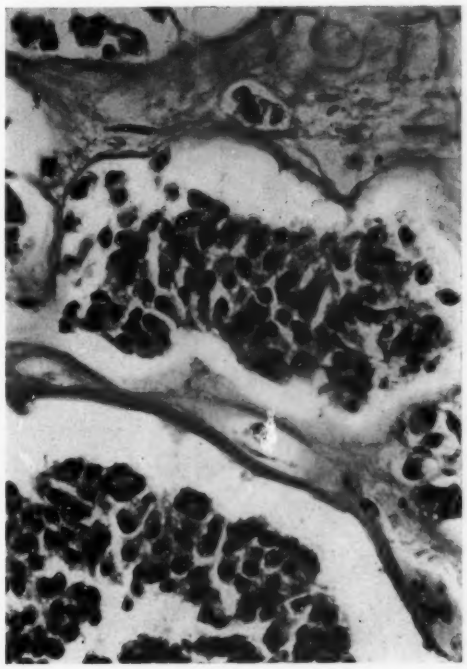

FIGURE VIIA

Father's large pancreatic tumour (×350); clumps of islet cells separated by hyaline fibrillary stroma. (Compare Figure VID)

Pathological Studies

The macroscopic features of the resected pancreas of this patient are summarized in Figure V.

At the first operation (March, 1960) the tail and portion of the body of the pancreas showed on the cut surface many round nodules slightly paler than the surrounding parenchyma and measuring from 2 to 7 mm. in diameter; one of the larger nodules was cystic and hæmorrhagic. Microscopically (Figures VIA and VIB) these nodules are composed of sheets, cords and acini of cells resembling those of the islets of Langerhans except for an increase in the size of the nuclei and the amount of cytoplasm. Presumptive evidence that these are non-beta cells in type is afforded by a negative response with Gomori's aldehyde fuchsin stain. The larger nodules have a capsule of fibrous tissue. In some areas mitotic figures can be found readily; cell groups can be found closely applied to capillary blood vessels, but no unequivocal evidence of vascular invasion has been found.

In addition to the nodules visible to the naked eye, there are changes to be found in a high proportion of

the islets of Langerhans in the intervening pancreatic parenchyma. In these islets the cells show enlargement and proliferative activity with loss of the normal islet pattern (Figure VIc).

At the second operation (August, 1960), a tumour weighing 36 grammes and measuring 5·5 by 3·7 by 3·0 cm. was found in the head of the pancreas. It was

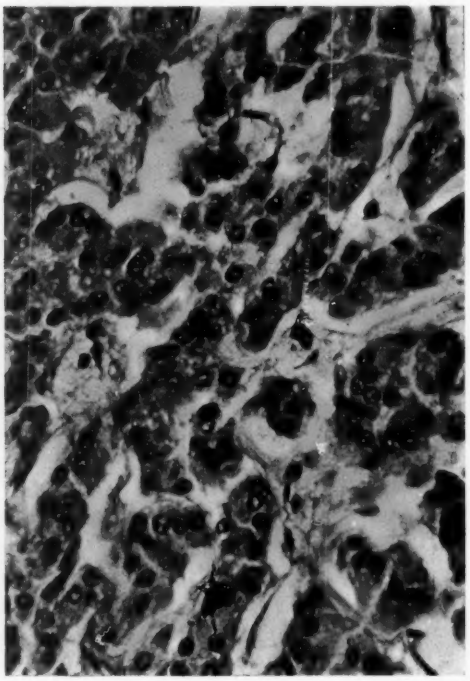

FIGURE VIIB

Same as Figure VIIA, same magnification ; branching columns of islet cells with a pattern similar to daughter's tumour. (Compare Figure VIB)

divided into four lobules of irregular size by delicate septa. Microscopically (Figure VID) the tumour is composed of compact clumps of uniform cells with round nuclei and faintly basophilic cytoplasm. The cell groups are separated by a small amount of vascular collagenous stroma. Cellular pleomorphism is minimal, and mitoses are difficult to find.

Elsewhere in the head and body of the pancreas there were two nodules, respectively 1·5 cm. and 1·0 cm. in diameter ; the microscopic structure of these and of the pancreatic parenchyma is similar to the pancreatic tissue removed at the first operation.

In summary, the pancreas contained one large and several small islet-cell adenomas, accompanied by widespread hyperplasia of the islet tissue, the latter being detected only at microcsopic examination.

The most striking changes at autopsy on the father of the patient were seen in the endocrine glands. In the tail, and encroaching on the body of the pancreas, there was a large tumour around which the splenic flexure of the colon was stretched and slightly kinked. This neoplasm was composed of clumps and cords of islet cells (Figures VIIA and VIIB) and closely resembled that found in his daughter.

The parathyroid glands were enlarged, the largest measuring 2 cm. in length, and microscopic examination showed proliferation of chief cells in the form of sheets, thick cords and acini (Figure VIIc). No water-clear cells or oxyphil cells were present, and adipose tissue was absent from the substance of the gland ; the chief cells were slightly larger than normal, and there was mild nuclear pleomorphism without a significant degree of mitotic activity. The features were those of parathyroid adenoma rather than hyperplasia secondary to renal disease.

A small chromophobe adenoma, histologically of diffuse type without sinusoidal arrangement (Figure VIID) was present in the anterior lobe of the pituitary gland, which was macroscopically slightly enlarged.

The adrenal glands were of normal size, and microscopically showed derangement of the cortex due to mild hyperplasia of the cortical cells without well-defined adenoma formation.

The necropsy revealed no evidence of metastatic new growth. An important negative finding was that the stomach, duodenum and small intestine appeared normal.

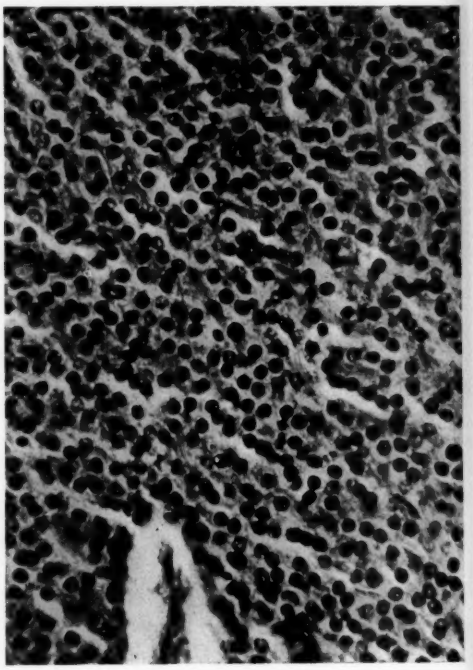

FIGURE VIIC

Father : parathyroid adenoma (×350)

DISCUSSION

Events in the years between 1954 and 1960 add further emphasis to the original comment made (Levitt and Saint, 1955) that " the patient is indeed fortunate to have survived

so many hazardous procedures and complications". The case is typical in all respects of the Zollinger-Ellison syndrome: the inexorable progression of peptic ulceration of jejunal mucosa despite the most radical gastric surgery, the occurrence of diarrhœa, either fatty or watery, and the finding of enormous gastric hypersecretion must always prompt a diagnosis of pancreatic islet-cell adenoma. In planning surgical treatment, the frequency of diffuse

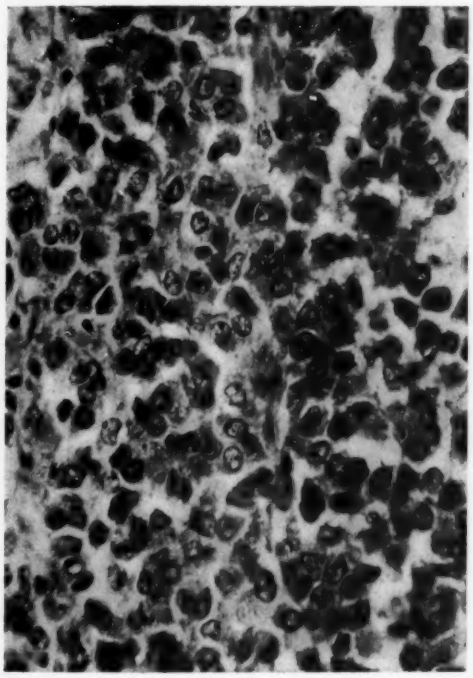

FIGURE VIID
Father: pituitary chromophobe adenoma ×350)

adenomatosis and the risk of malignant change would seem to render total pancreatectomy a mandatory procedure. Comprehensive investigation of the patient with duodenal ulcer should include a quantitative study of overnight gastric secretion as well as the conventional histamine test meal, for it would appear that islet lesions are not excessively rare; Ellison, Abrams and Smith (1959) found 26 instances of islet abnormalities (hyperplasia or discrete adenoma) in 812 autopsies on subjects having had gastroduodenal ulcer. In particular, adenomatosis must always be suspected in cases of ulceration at unusual sites in the duodenum or jejunum.

A few observations suggest that gastric hypersecretion and hyperacidity are not the sole factors involved in the production of peptic ulcers in these cases. Ulceration recurred, in this patient, in the presence of a very limited area of functioning gastric mucosa. It may be presumed that functioning adenomas of the pancreas have been present since birth, yet symptoms of ulceration were first manifest at the age of 20 years. Her father, who had identical pathological lesions in the pancreas, escaped throughout his life the complications of peptic ulceration. These considerations are of practical as well as of theoretical importance. The case for total pancreatectomy has been argued above; if the surgeon is not emboldened to undertake this most radical procedure, then in view of the risk of leaving behind microscopic adenomas he is at least committed to removing the whole of the stomach.

This unusual case casts light, parenthetically, on two aspects of alimentary physiology. First, we have noted that the production of intrinsic factor in sufficient quantity to maintain normal serum levels of vitamin B_{12} was located precisely in a few square centimetres of gastric mucosa lying immediately distal to the cardio-œsophageal junction. Secondly, studies were incidentally made on intestinal absorptive function in the presence of the unusual combination of total gastrectomy and total pancreatectomy. Absorption of fat and fat-soluble vitamin is grossly impaired: large doses of pancreatic extract (four tablets of "Pancrex" with meals four times a day) have failed to lower the fæcal fat content below 50%. The patient is presumably in negative protein balance (diminishing weight and low serum albumin level). Xylose absorption is grossly impaired for reasons not clearly understood. Intestinal hurry is clearly not a major causative factor, for barium studies have shown the efferent jejunal loop to be acting as a substitute hopper, and follow-through times are slightly above normal.

It is the genetic and pathological features of the case which perhaps excite the greatest interest. In addition to pancreatic adenomatosis, the patient has almost certainly a pituitary chromophobe adenoma, and very probably parathyroid adenomas or hyperplasia (the hypophosphatæmia and occasional hypercalcæmia being perhaps indicative). Evidence of adrenal cortical hyperplasia or adenomatosis is not forthcoming. All four endocrine lesions were present in the father.

Moldawer, Nardi and Raker (1954) reviewed 28 cases of multiple adenomatosis; in most

instances the pancreatic lesion comprised a beta-cell adenoma. It is of interest that in eight of these 28 cases mention was made of peptic ulceration as a complication. Fewer instances of the association of alpha-cell pancreatic with other endocrine adenomas have been reported (Cunningham, Howe and Evans, 1952; Underdahl, Woolner and Black, 1953; Wermer, 1954; Ellison, 1956; Zubrod *et alii*, 1958); the cases reported by Cooke *et alii* (1960) and by us would seem to be the seventh and eighth respectively to be described.

The genetic aspect of endocrine adenomatosis is well documented, Underdahl, Woolner and Black (1953) reporting eight cases of familial adenomatosis and referring to 13 others culled from the literature. The mode of inheritance appears to be dominant; Wermer (1954) reported a family in which the father and five of nine offspring had adenomas of pituitary, parathyroid, adrenal cortex and pancreatic islets in various permutations. Presumably the gene responsible influences the differentiation of those parts of the foregut entoderm destined to form ductless glands—anterior pituitary lobe, parathyroids and pancreatic islets. A possible explanation of the occurrence of adrenal cortical hyperplasia or adenoma in this constellation is that in man some of the cellular components of the adrenal cortex may be of entodermal origin.

ACKNOWLEDGEMENTS

We are grateful to the Deputy Commissioner of the Repatriation Department for permission to study the records of one of our patients, and to members of the Department of Clinical Biochemistry and the Department of Hæmatology for their assistance. The photographs were prepared by Mr. H. Upenieks.

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A Review Written by Request

ARTHROPOD-BORNE VIRUS DISEASES OF MAN

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MOSQUITOES were shown to be required for the transmission of certain viruses only a few years after the first demonstration that some of the agents we now call viruses could infect animals. The earliest demonstration that a mosquito was required for the transmission of a virus was made by the U.S. Yellow Fever Commission in 1901 (Reed *et alii*, 1911). In 1906 Bancroft, in Australia, produced evidence that dengue was transmitted by *Aëdes ægypti*, and his work was later confirmed by Cleland *et alii* (1916, 1919). Doerr and Russ in 1909 showed that phlebotomus (sandfly) fever was transmitted by the insect *Phlebotomus pappataci*. In these two last-mentioned cases, although it was clearly shown that the disease was of virus origin, the responsible virus was not isolated until many years after the transmission had been worked out. In the case of Australian X disease, the virus was isolated many years before its association with a mosquito vector was proven. In 1917 Cleland and Campbell reported on the isolation of a virus pathogenic for monkeys from the epidemics of X disease, and they reported further on this virus in 1919.

In recent years there has been a great expansion of work on these viruses, and essentially two types of investigation have been made. In the first type the workers have examined large numbers of mosquitoes and wild animals for the presence of viruses and have later made investigations to discover whether any of the viruses so isolated were of significance in the disease of man and domestic animals. The other main type of investigation has been that of seeking the causes of known outbreaks, or of sporadic cases of disease in man and domestic animals, and either the characteristics of the virus discovered or the epidemiology of outbreaks of the disease has led to examination of the possibility that these were arthropod-borne infections. In some cases serological investigations, which have shown the existence of antibodies to known arthropod-borne viruses in man or domestic animals, have led to further investigations to isolate those viruses and to find their actual relationship to known viruses. By now, between 125 and 150 arthropod-borne viruses have been described, and 51 of them are known to be pathogenic for man.

In 1960 the World Health Organization convened a study group on arthropod-borne viruses, and this group gave the following definition :

> An arthropod-borne animal virus is defined as one which, in nature, can infect hæmophagous arthropods by their ingestion of infected vertebrate blood. It multiplies in their tissues and is transmitted by bite to susceptible vertebrates. Viruses which are otherwise transmitted by arthropods, e.g. Myxomatosis and Avian pox viruses, are thus excluded from this group.

GROUPING OF ARTHROPOD-BORNE VIRUSES

The classification of arthropod-borne viruses rests at present entirely on serological relationships. These relationships have been mainly studied by means of the virus-neutralization test and the complement-fixation test, and by the inhibition of viral hæmagglutination by immune sera (hæmagglutination-inhibition test). In some instances cross-protection tests in animals have proved valuable in distinguishing viruses which appeared closely related by virus neutralization. In the main the present classification has been based on the first three tests. By these means the known viruses have been grouped into four major groups known as as A, B, C and Bunyamwera, and nine further small groups with two to four members. A number of viruses, which show no serological relationship to any of the others included as arthropod-borne, remain ungrouped. The investigations so far have not shown any virus to have any serological relation to more than one group.

At least in groups A and B the hæmagglutination-inhibition test is the most broadly group-reactive and shows the group relations more clearly than the other tests. The complement-fixation test is also quite broadly reactive and, in the case of group C, covers the group better than the hæmagglutination-inhibition test (Casals and Whitman, 1961). Casals (1957), who has been mainly responsible for finding the basis for the present classification of arthropod-borne viruses, has shown that there are important differences in the specificity of the neutralization tests under differing conditions. In a human being who has had only a single infection, or in an animal early in the course of immunization, there is a highly specific response which may give no cross reaction, or a cross reaction only to a very low titre with related viruses. Later in immunization, or in individuals who have had more than one infection with an arthropod-borne virus of the same group, even if the repeated infection has been with the same virus, sera show much less specificity in their response to any test. When the broadly-reactive hæmagglutination-inhibition test is used, it may be impossible to decide from such sera which virus of a particular group was responsible for the most recent infection. In the Bunyamwera group (Casals and Whitman, 1960), the neutralization test is less highly specific than in the other groups, but even in this group there is a great deal of crossing by hæmagglutination inhibition. Groups A and B are the two largest groups, and subgroups have been demonstrated within them. Viruses inside the subgroup are more closely related one to the other than they are to other members of the group (Casals and Reeves, 1959).

SYNDROMES CAUSED BY ARTHROPOD-BORNE VIRUSES IN MAN

Most of the viruses of great importance in human disease are members of groups A and B. In group A are the three forms of equine encephalitis, of which the eastern form has been recorded from the Philippines (Table I), although the others have not yet been found outside the American continent. The known cause of the syndrome of fever, malaise, headache, arthritis and rash, Chikungunya virus, is a member of this group. Very recently another virus closely related to this, but transmitted by an anopheline instead of a culicine mosquito, has been responsible for at least three-quarters of a million cases of this syndrome in East Africa. This virus has been given the name of O'nyong nyong (Williams and Woodall, 1961). In Thailand a very severe syndrome of fever, headache, general and local pains, prostration and hæmorrhage has been associated with a virus closely related to or identical with Chikungunya (BAH 306). In Australia a syndrome of polyarthritis with rash has been associated with antibodies to different group A viruses, AMM 2354 and AMM 2021 (Halliday and Horan, 1943; Anderson and French, 1952; Doherty *et alii*, 1961). So far no virus has been isolated from the Australian cases, but it does not seem very likely that the virus is identical with AMM 2354. In Malaya, where this virus was isolated, neither it nor AMM 2021 has yet been associated with any syndrome.

Group B contains a large number of viruses important for man, and of these important viruses at least three are, or have been, present in Australia. The most important of all the arthropod-borne viruses is that of yellow fever, which has so far been recorded only from the African and American continents, but since the main urban vector is present in the tropics and subtropics throughout the world, it would be quite possible for this virus to appear in any country, although it is unlikely to establish itself in the absence of a large wild-monkey population. Antibodies have been demonstrated against Zika virus, which belongs to the yellow fever subgroup of the group B, in India, Malaya and Borneo, and, therefore, the possibility arises that infection with Zika virus, which is ordinarily responsible for an influenzal type of syndrome, may eventually reach Australia or New Zealand. The dengue viruses I and II have both been responsible for outbreaks of dengue in Australia, and the epidemiology in Australia will be discussed more fully later in this article. Type III has been responsible for the very severe syndrome described as hæmorrhagic dengue in the Philippines and Thailand and type IV for the same syndrome in the Philippines, but it has not yet been described elsewhere. These viruses have only recently been isolated, and their distribution may well be much wider than we know at present. West Nile virus also causes a dengue-like syndrome, but this virus has not yet been demonstrated nearer than South India. Like group A, group B contains a number of viruses capable of causing encephalitis in man. The most important of these is Japanese B encephalitis, which, in the countries in which it occurs, is responsible for a very large number of cases of severe and often fatal encephalitis. Murray Valley encephalitis virus, which is the member of the Japanese B subgroup found in Australia and New Guinea, was undoubtedly the agent responsible for the outbreaks of X disease in 1916–1917, 1917–1918, 1922 and 1924–25, as well as for the 1951 epidemic and

for the three cases occurring in the Murray Valley in 1956 (Anderson *et alii*, 1958).

All the viruses which have been mentioned above are transmitted by mosquitoes, but there is a further subgroup of serologically closely related viruses in group B which are transmitted by ticks. These produce encephalitis or meningo-encephalitis or a hæmorrhagic syndrome. These viruses have been mainly studied in Europe and Asiatic Russia, but very recently an encephalitis-producing virus has been shown to exist in Canada and the United States. It was first isolated from a case of encephalitis in Toronto (McLean and Donohue, 1959). In 1955 a new epidemic disease appeared in the Kyasanur Forest in Mysore State in South India (Work *et alii*, 1957), and epidemics have continued to occur every year since then in a slowly-extending area. This has proved to be a hæmorrhagic fever due to a member of this group. The nearest known focus of a similar syndrome due to a closely related virus is Omsk in the U.S.S.R. This obviously raises the possibility that migrating birds may be very important, either in carrying the virus itself or in carrying infected ticks to spread such diseases to new areas. A similar virus (TP 21) has been isolated from a tick in Malaya (Smith, 1956), but no cases of human disease due to this virus have been recorded as yet.

Viruses of group C have so far been found only in Brazil, where they are responsible for an

TABLE I

Arthropod-Borne Viruses Known to be Pathogenic for Man Found in India, South-East Asia, Indonesia, Australasia or the South-West Pacific Area

Syndrome	Virus	Group	Isolation in Nature from			Vector	Areas where Virus has been Isolated or Disease Proved to Occur	Antibodies only Found	References
			Human	Vertebrate	Arthropod				
Fever with malaise, headache and pains, of generalized and localized distribution	Zika	B	+	+	+	Mosquito	Nigeria, Uganda	India, Malaya, Borneo	Dick *et alii*, 1952; MacNamara, 1954; Smithburn *et alii*, 1954; Smithburn 1954
	Bunyamwera	Bunyamwera	+		+	Mosquito	East and South Africa	Borneo	Smithburn, Haddow and Mahaffy, 1946; Kokernot *et alii*, 1958; Smithburn, 1954
Fever with malaise, headache, joint pains and rash	Chikungunya	A	+		+	Mosquito	East and South Africa	See note[1]	Ross, 1956; Gear and Reed, 1957
Fever with headache, general and localized pains, rash and lymphadenopathy	Dengue type I	B	+			Mosquito	South and South-east Asia, Oceania and Pacific		Sabin, 1952
	Dengue type II	B	+			Mosquito	Circumglobal in the tropics		Schlesinger and Frankel, 1952; Anderson *et alii*, 1956
	West Nile	B	+	+	+	Mosquito	Africa, Near East, India		Bernkopf *et alii*, 1953 Taylor *et alii*, 1956
Fever with headache, generalized and localized pains, prostration and hæmorrhagic signs	BAH 306, TH 35 (probably identical with Chikungunya)	A	+			Mosquito	Thailand		
	Dengue type III	B	+			Mosquito	Philippines, Thailand		Hammon, 1960
	Dengue type IV	B	+			Mosquito	Philippines		Hammon, 1960
	Kyasanur Forest disease	B	+	+	+	Tick	South India		Work *et alii*, 1957 Work, 1958
Acute febrile illness with encephalitis	Eastern equine encephalitis	A	+	+	+	Mosquito	North and South America, Philippines		Schaeffer *et alii*, 1954; Donaldson, 1958 *et alii*†
	Japanese B	B	+	+	+	Mosquito	China, Japan, South-east Asia, India, Philippines		Tanaguchi 1936; Sabin, 1950; Work and Shah, 1956
	Murray Valley	B	+		+	Mosquito	Australia, New Guinea		French, 1952; Miles, 1952; French *et alii*, 1957

[1] In Australia this syndrome has been associated with antibodies to another group A virus, AMM 2354. No virus has yet been isolated from the Australian cases and AMM 2354 has not itself been associated with any disease in man.

influenzal type of syndrome. A similar syndrome is caused by some members of the Bunyamwera group, which are much more widely spread. Antibodies against Bunyamwera virus have been demonstrated in Borneo, although not in India or Malaya. Viruses of other groups, and some of the ungrouped viruses, such as phlebotomus (sandfly) fever and Rift Valley fever, are important causes of human disease in the parts of the world in which they occur; but like yellow fever, they have not yet been demonstrated anywhere in India, South-East Asia, Indonesia, Australasia or the South-West Pacific region. Table I lists the viruses, known to be human pathogens, which either have been shown to cause disease in this area, or against which neutralizing antibodies have been demonstrated there. The nature of the evidence for their presence is also given, together with the sources from which they have been isolated, the type of vector and the syndrome with which each virus has been associated. Every type of syndrome known for arthropod-borne viruses, with the exception of the yellow fever syndrome, may be found in this region. This selection has been made on the grounds that these viruses, or viruses related to them, are those most likely to be found in Australia and New Zealand or to be brought to us either by migrating birds or by men. The rest of this review will deal with viruses and syndromes known in Australasia.

Dengue

The syndrome of dengue appears to have been described towards the end of the eighteenth century, and reports on many epidemics were published during the nineteenth century. The name "dengue" was accepted by the Royal College of Physicians of London in 1869, and has been in standard usage since then. Work done in Australia was responsible for establishing this as a disease normally transmitted by the mosquito *A. ægypti*. The first paper was published by Bancroft in 1906, and later work by Cleland, Bradley and MacDonald published in 1916 and 1919 conclusively proved that this mosquito was the main vector of the disease. Very large epidemics of dengue have occurred in Australia, and in the Queensland and New South Wales epidemic of 1925–1926 there were approximately 560,000 cases. A further large epidemic occurred in 1942–1944 (Lumley and Taylor, 1943).

It was not until World War II that the virus was finally isolated from the blood of human patients (Sabin and Schlesinger, 1945). By the time when the virus had been satisfactorily adapted to the laboratory mouse, its virulence for man had become so attenuated that it was practicable to use this strain as a vaccine, although a proportion of the vaccinated showed a pyrexial reaction and mild malaise. It was quite soon shown that there were two serological types of dengue virus and that an attack from one did not confer immunity from the other (Sabin, 1950*a*). The type strain of the first isolated came from Hawaii and of the second from New Guinea, and these strains are now known respectively as dengue type I and type II. Hammon (1960) has recently isolated two further closely related viruses, which are now called dengue types III and IV. The former has been isolated both in Thailand and in the Philippines and the latter in the Philippines only. Both these viruses cause a very severe syndrome termed "hæmorrhagic dengue", and in the Philippines outbreak there were 15% of deaths out of 750 cases reported in Manila (Hammon *et alii*, 1958). So far there is no evidence that types other than I and II have been present in Australia.

The most recent epidemic of typical clinical dengue in Australia was that which occurred in 1953–1955 in North Queensland, when in the Townsville area approximately 15,000 people out of a total of 40,000 were affected (Rowan, 1956). In this epidemic the city of Brisbane, from which *A. ægypti* has been eliminated, escaped. Brisbane had had a high incidence in previous Queensland epidemics (Doherty, 1957). Doherty and Carley have made serological investigations to attempt to establish what types of dengue virus have been responsible for the major epidemics in Queensland. Their results lead to the conclusion that the epidemics of 1925–1926 and 1953–1955 were due to type I virus, while the 1942–1944 epidemic was predominantly due to type II, but some cases were due to type I. No evidence has been found of dengue infection in Brisbane since 1944, and no evidence has been found of further cases in the Townsville region since the end of the epidemic in 1955. Children born since 1955 have no antibodies against dengue virus.

The situation in the Northern Territory is less clear. Prior to World War II, dengue was said to be endemic and occasionally epidemic in Darwin. New arrivals in the town usually suffered from a typical attack of dengue shortly after arrival, while old residents who had been away temporarily did not suffer similarly on their return. Epidemics of dengue sometimes spread as far as latitude 20° south or possibly further. Darwin obtained a reticulated water supply in 1940, and after this the army destroyed all domestic water tanks and undertook a vigorous anti-mosquito campaign which

eliminated *A. ægypti*. This mosquito was said not to have returned to Darwin by 1954 (Miles and Dane, 1956). However, in 1958 an epidemic occurred on Croker Island off the Arnhem Land coast and at Oenpelli Mission on the western borders of Arnhem Land, in which the patients were affected by an illness with pyrexia lasting from seven to ten days, generalized aches and pains, backache, cough and sore throat. The severe backache and rash characteristic of dengue were not encountered during this epidemic. Strong serological evidence was obtained that this outbreak was due to dengue type I virus (McLean and Magrath, 1959). These last findings again raise the possibility, which has been widely discussed on many occasions, that dengue has an animal reservoir in which it survives between the major human epidemics. While this theory is very attractive to explain such findings as that of endemicity in the sparsely-populated Northern Territory of Australia, there is little evidence for it, and the elimination of clinical dengue from Darwin at the time when *A. ægypti* was eradicated must be regarded as circumstantial evidence against this theory. However, there is some serological evidence suggesting a possibility of animal infection in both birds and flying foxes (*Pteropodidæ*) (O'Connor *et alii*, 1955; Doherty *et alii*, 1959).

There is very good serological evidence of the existence of group B viruses in the South Island of New Zealand, in Fiji and in Western Samoa. In Fiji and Western Samoa it seems certain that dengue viruses I and II are or have been present. There may be another virus also present in the Fiji group, and it seems probable that the virus present in the South Island of New Zealand is not a member of the dengue group, since antibodies are mainly found in birds rather than in man (Maguire, 1960; Miles, Maguire and Ordish, 1960; Miles and Maguire, 1960; Miles, Ross and Maguire, unpublished data).

Polyarthritis and Rash

Late in 1942 and at the beginning of 1943 there was an epidemic of polyarthritis among troops in the Northern Territory of Australia, which was described in detail by Halliday and Horan (1943). The disease was an acute febrile illness lasting one to four weeks, and characterized by a fleeting exanthem, mild fever, pain, and in the more severe cases swelling of joints and tender enlargement of the lymph glands. The disease ran a benign course and recovery was complete. The severe constitutional symptoms common in dengue were not observed in this outbreak, during which 105 cases were admitted to the army hospital where observations were made. In the late summer and early autumn of 1956, an epidemic of a similar syndrome occurred in the Murray Valley, and it is estimated that in Mildura there were between 1000 and 2000 cases of the disease in a population of 20,000 persons (Anderson and French, 1957). In this outbreak the disease was uniformly mild, and the patients usually had no fever. The rash was profuse and similar to that described by Halliday and Horan; but Horan, after examining colour photographs of these patients, said that, unlike the Murray Valley patients, his patients had had no lesions on the face or the palate, and that in the Northern Territory outbreak the individual lesions had been more widely separated than in the Mildura cases. A similar condition has been described from Narrandera in 1928 (Nimmo, 1928*a*, 1928*b*). Rather earlier in the same season there was a similar epidemic in Hay (Edwards, 1928). Nimmo (1946) has stated that he has seen the same syndrome during a wet season in the Torres Strait area. Since the Murray Valley outbreak there has been a similar outbreak in the south-west of Western Australia, and Doherty *et alii* (1961) have also reported on the development of antibodies to AMM 2354 in individuals with this syndrome in and around Brisbane. Anderson *et alii* (1961) state that there have been ten major epidemics of polyarthritis reported in Australia and nearby northern islands since 1928.

Shope and Anderson (1960) showed that this syndrome was associated with the development of antibodies against the group A arthropod-borne viruses AMM 2354 and AMM 2021, which were originally isolated from mosquitoes in Malaya, and which had not yet been clearly associated with any particular syndrome. The paper of Shope and Anderson, together with the work of Anderson *et alii* (1961) and work carried out by Stanley and Choo in Western Australia, has shown that antibodies to AMM 2354 are very widespread in Australia and New Guinea. The antibodies were found in human, horse, pig and cattle sera, but there were no antibodies in the sera of domestic chickens and no significant antibodies in small groups of sheeps, cats, dogs, goats and sundry species of wild animal.

Despite considerable efforts to isolate virus both from patients and from mosquitoes, no isolations have yet been made from the Australian cases. Recently, Doherty *et alii* (1961*b*) have isolated a virus closely related to Sindbis virus, a group A virus not yet associated with any syndrome in man, from the Carpentaria

region, but it is clear that this virus is not the one responsible for the AMM 2354 antibodies. Two group A viruses are known to produce polarthritis with rash. These are Chikungunya virus and O'nyong nyong virus. In both these infections the constitutional symptoms in the patients are usually severe, and the joint pain would appear to be very much more severe than has been usual in the Australian outbreaks. The native name O'nyong nyong means " joint crusher " and gives some idea of the symptoms of the patients. The syndrome produced by the virus closely related to Chikungunya virus recently isolated in Thailand is an extremely severe hæmorrhagic fever, similar to hæmorrhagic dengue and with a high mortality. Fortunately the Australian outbreaks do not bear much resemblance to this. The clinical picture and the serological results suggest that the Australian virus is different from the known viruses producing polyarthritis and rash in other countries.

Only a very limited number of sera from New Zealand and South-Western Pacific Islands have been tested for antibodies against AMM 2354. All the tests done were negative.

Murray Valley Encephalitis and Australian X Disease

Epidemics of acute encephalitis were reported from south-eastern Australia and Queensland in the summers of 1916–1917, 1917–1918, 1924–1925 and 1950–1951. A further epidemic occurred in Queensland in 1922, and there were three cases in the Murray Valley in 1956 (Cleland and Campbell, 1917, 1919; Kneebone and Cleland, 1926; Anderson, 1952, 1954; Anderson *et alii*, 1958). The outbreaks before 1951 were termed Australian X disease, and since the 1951 outbreaks and the reisolation of virus the disease has been known as Murray Valley encephalitis (MVE). The reasons for regarding these various outbreaks as due to the same agents have been discussed by various authors. The close similarity of the epidemiology of the different outbreaks has been discussed by Miles and Howes (1953) and by Anderson and Eagle (1953). Serological studies on individuals from the Broken Hill area by McLean and Stevenson (1954) gave additional evidence of identity, and Anderson (1954) has reviewed the reasons for regarding these two diseases as the same. Numerous serological studies have been undertaken to obtain evidence on the areas in Australia and New Guinea which have been infected with this virus. The results clearly show that most of the mainland of Australia and New Guinea, apart from the central highlands, have experienced a spread of this or closely-related viruses during quite recent years. The results indicate that, in general, infection has been more general and more frequent in the tropical north of Australia, where there is a reliable monsoonal rainfall, than in the rest of Australia. The positive results are shown in Table II.

TABLE II

Known Group B Virus Activity in Australia and New Guinea, 1951–1960

Year	Areas where Activity was Reported	Virus	Nature of Report		
			Proved Cases	Serological Findings	Virus Isolation
1951	Murray Valley[1,2]	MVE	+	+	+
	Coastal Queensland[1]	MVE or closely related		+	
	Gulf of Carpentaria[1]	MVE or closely related		+	
	Northern Territory[3]	MVE or closely related		+	
	New Guinea[1]	MVE or closely related		+	
May, 1953–December, 1954	Northern Territory[4]	MVE or closely related		+	
1953–1955	North Queensland[5,6]	Dengue type I	+	+	
February, 1954–May, 1955	Carpentaria[7]	MVE or closely related		+	
February–March, 1956	Murray Valley[8]	MVE	+	+	
	Papua–New Guinea[9]	MVE	+	+	+
August, 1957–July, 1958	New Guinea[12]	MVE or closely related		+	
June, 1958–July, 1959	Northern Territory[12]	MVE or closely related		+	
November, 1958–July, 1959	Carpentaria[10]	MVE or closely related		+	
March–April, 1960	Carpentaria[11] (Mitchell River Mission)	MVE		+	+
		Other group B viruses		+	+

[1] Anderson *et alii* (1952).
[2] Miles and Howes, 1953.
[3] Beech *et alii*, 1953.
[4] Miles and Dane, 1956.
[5] Doherty and Carley, 1960.
[6] Rowan, 1956.
[7] Ludford and Cook, 1957.
[8] Anderson *et alii*, 1958.
[9] French *et alii*, 1957.
[10] Doherty *et alii*, 1960.
[11] Doherty *et alii*, 1961a.
[12] Anderson *et alii*, 1961.

MVE virus is regarded as having an enzootic cycle between wild birds and mosquitoes, and therefore it is very difficult to be absolutely certain that the virus has not been active in a particular area in any season. However, at least for the Murray Valley region, there is very good evidence of complete absence of the virus in certain seasons. In 1952 very extensive mosquito collections were made in the Murray Valley in a year in which no cases of disease were found. No virus resembling MVE was isolated from 17,833 mosquitoes (Reeves *et alii*, 1954). After the small outbreak in the Murray Valley in 1956, during the summer of 1957 large mosquito collections were again made, and 102 sera from domestic fowls were examined. Again no evidence was found of virus activity in the area in the 1956–1957 summer (Anderson *et alii*, 1958). Further, although the doctors in the Murray Valley area are well aware of this syndrome and on the lookout for any cases, no suspicious cases have been found except in those years in which virus activity has been proven. The evidence is therefore very good that this virus is not enzootic in the Murray region, but appears in occasional epidemics.

By contrast, in the Northern Territory and North Queensland most investigations have given evidence of recent virus infection. The only exception is the observation of Doherty *et alii* (1959) that all of 15 native children aged between two and three years at Mitchell River Mission from whom blood was taken in 1958 were without antibodies to MVE virus. It is, of course, possible that, like eastern equine encephalitis virus, MVE has a wild-bird-mosquito cycle, in which the main mosquito vector is a strictly avian mosquito reluctant to bite any mammals. Our present lack of knowledge of the feeding habits of Australian mosquitoes makes it impossible to do anything more than speculate on this. Up to the present, MVE virus has been isolated from only two species of mosquito, *Culex annulirostris*, which is an indifferent feeder and thought to be the main epidemic vector in the Murray Valley, and *A. normanensis* (Doherty *et alii*, 1961*b*). It is now generally held that MVE virus is enzootic in a wild-bird-mosquito cycle in Northern Australia and/or New Guinea. Miles and Dane (1956) suggested that, in those limited areas of permanent swamp which exist in northern Australia, the virus might survive in a mosquito-bird cycle. They pointed out that as, during the dry season, more and more water-holes and swampy areas dried out, an increasing number of birds would be forced to visit the few permanent swamps. As a result, it would be quite possible for a continual supply of susceptible birds to be brought to a small enzootic focus throughout the year and so enable the virus to survive in a simple bird-mosquito cycle. It was then suggested that, in years when the conditions were particularly good for both bird and mosquito breeding early in the season in the enzootic area, a very high concentration of infected birds built up, and that as they dispersed from the breeding area, they spread the infection. In suitable conditions they might bring it to the settled areas.

All the years in which large outbreaks have occurred in southern Australia have been associated with exceptionally heavy spring rainfall in northern Australia, but the three cases in 1956 which were associated with quite considerable virus activity in the Murray Valley were not associated with such conditions. This suggests that the theories associating spread of MVE virus with very closely defined meteorological conditions (Miles and Howes, 1953; Anderson and Eagle, 1953) may need some modification. Our present knowledge of the epidemiology of the arthropod-borne viruses which cause epidemic encephalitis in man have recently been reviewed in detail by Miles (1960).

Other Viruses

Recent work in Queensland has revealed the presence of a number of arthropod-borne viruses in the Carpentaria region. Doherty *et alii* (1961*a*, 1961*b*) have isolated numerous viruses from a collection of mosquitoes made in March and April, 1960, in the Mitchell River Mission region in the Gulf of Carpentaria. As well as their isolations of Murray Valley encephalitis virus which have been mentioned, two further types of group B virus were also isolated, which appeared to be distinct from any of the known serological types. There is serological evidence that at least one of these two types (Kunjin) infects man in the Mitchell River area, but no syndrome has yet been associated with it (Doherty *et alii*, 1961*a*).

Four further distinct virus types were isolated from the same batch of material. One of these is similar to, or identical with, the group A virus " Sindbis ", which has not yet been shown to cause any disease. This virus has also been isolated in Africa and India (Taylor *et alii*, 1955; Weinbren *et alii*, 1956). The other three do not fall into any of the recognized groups, although two are related to one another. Six strains of a further virus which has been shown to infect domestic fowls were also isolated. This virus does not possess all the usual physical characters of an arthropod-borne virus, and will not necessarily prove to be a member of the group (Doherty *et alii*, 1961*b*).

CONCLUSION

In this brief review, an attempt has been made to list those arthropod-borne viruses that cause disease in man and may possibly be found in Australia or New Zealand, and to give a rather more detailed discussion on what is known of the arthropod-borne diseases that are present in Australia and New Guinea. It is clear that our knowledge is very far from complete on any of these conditions. We have some information on Murray Valley encephalitis and dengue, from which virus strains have been isolated, but we do not even know with certainty what virus is responsible for the syndrome of polyarthritis and rash, although we can be confident that it is due to a group A virus related to AMM 2354. The new viruses recently isolated in North Queensland are viruses which have not yet been shown to be associated with any syndrome, although there is evidence that some of them can infect man. The assessment of their importance must await further investigations. So far as New Zealand is concerned, the only evidence so far of the existence of arthropod-borne viruses is serological, and whether these viruses are of any significance in human disease is still unknown. In the Pacific Islands it is clear that dengue viruses are present as endemic viruses or during occasional epidemics. There is suggestive evidence that other viruses also may be present, but this evidence is entirely serological. A great deal of further work on this group of viruses will be required before a clearer picture can be given.

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AUSTRALASIAN MEDICAL PUBLISHING CO. LTD.
SEAMER AND ARUNDEL STS., GLEBE, SYDNEY

AUSTRALASIAN ANNALS OF MEDICINE

NOVEMBER 1961

Registered at the General Post Office, Sydney, for transmission by post as a periodical

WHOLLY SET UP AND PRINTED IN AUSTRALIA
BY AUSTRALASIAN MEDICAL PUBLISHING
COMPANY, LIMITED SEAMER STREET, GLEBE
NEW SOUTH WALES.

VOLUME 10 NOVEMBER 1961 NUMBER 4

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